The Archives of Internal Medicine

Vol. XV NOVEMBER, 1914 No. 5

THE PULSE-FLOW IN THE BRACHIAL ARTERY

IV. REFLECTIONS OF THE PRIMARY WAVE IN DICROTIC AND MONOCROTIC PULSE-FORMS *

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It is well known that in most tracings from the peripheral arteries various secondary waves follow the principal or primary wave on the descending limb of the pulse. The chief of these is usually spoken of as the dicrotic wave; and when this wave can be distinctly felt by the palpating finger on the radial artery, the pulse is described as dicrotic. The typical dicrotic pulse is relatively common in typhoid fever, but it occurs not infrequently in all types of infection. It is also occasionally encountered in other conditions, as, for example in cardiac patients with badly broken compensation. Marked instances of dicrotism are frequently but not invariably associated with low blood-pressure. On the other hand, equally low blood-pressures occur without marked dicrotism. In this as in many other conditions the relation between blood-pressure, whether systolic or diastolic, and pulse form is but a loose one. Pulse form does not depend directly on blood-pressure, though the two are controlled by many factors in common.

During the past year I have studied the brachial flow in seven patients who presented definitely palpable dicrotic pulses. In all of these cases the records obtained showed characteristic variations from the usual pulse-flow in the brachial artery (Figs. 1 and 2). Instead of the step-like entrance of blood most commonly seen in normal tracings, the primary wave was immediately followed by a marked retrograde movement of blood in the artery, and this in turn was followed by a forward movement of blood due to the dicrotic wave. The smaller secondary waves so frequently seen on the normal pulse were absent. The tracings showed many points in common with those obtained from normal individuals who have taken nitroglycerin. The marked backflow in the brachial artery which occurs just after the entrance of the primary wave is present in both, and is, I believe, due

* Submitted for publication June 12, 1914.
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to a reflection of the primary pulse-wave in the vessels of the arm. Certain differences between the nitroglycerin pulse and the dicrotic pulse of fever will be discussed later.

One of the characteristic features of sphygmographic radial tracings of the dicrotic pulse is the marked fall in the pressure-curve between the primary and the dicrotic waves, which may be so great that the tracing descends nearly as low as and occasionally even lower

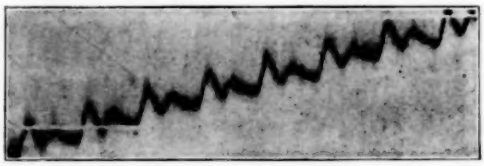

Fig. 1.—Common type of pulse flow in a palpable dicrotic pulse. The horizontal lines represent a difference in volume of 2 c.c. Vertical interruptions, ¼ second. Rate of flow, 2.1 c.c. per hundred c.c. arm substance per minute. Height of primary wave above the average rate of flow, 0.5 c.c.

than it does at the end of the pulse-cycle. Our records show that this fall of pressure is associated with a backflow in the brachial artery. Hirschfelder[1] has based his classification of pulse-form on the degree of this fall after the primary pulse-wave, and Marey apparently assumes a similar classification. According to this view, all pulses which show a marked fall in the late systolic portion of sphygmo-

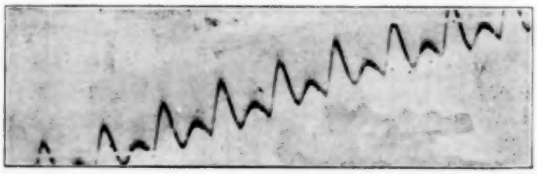

Fig. 2.—Unusually large primary wave (0.8 c.c.) with unusually marked backflow after this wave in a patient with dicrotic pulse. Rate of blood flow, 2.2 c.c.

graphic tracings are grouped together, irrespective of the size of the dicrotic waves. In the present paper I shall designate as monocrotic a pulse which shows this marked fall of pressure in late systole without the presence of a prominent dicrotic wave. That this sudden fall of pressure is of greater importance than is the size of the dicrotic elevation is shown by the fact that variations in the latter often occur while the former remains constant. Thus von Kries[2] found that during

1. Hirschfelder, A. D., Diseases of the Heart and Aorta, 1910, p. 44.
2. Von Kries: Studien zur Pulslehere, Freiburg, 1892.

the height of amyl nitrite action, the radial pulse was monocrotic and that the dicrotic wave appeared and became more marked as the action of the drug wore off. Febrile patients may also show great variations in the size of the dicrotic wave while preserving the marked systolic fall, as may be seen in tracings published in Marey's "Circulation du sang" (Fig. 274). Further evidence of the intimate relationship which exists between the monocrotic pulse as above defined and the dicrotic pulse is also obtained by records of the brachial pulse flow. A typical monocrotic pulse is shown in Figure 3, and it will be seen that like the dicrotic pulse there is a very marked backward movement of the blood in the brachial artery just after the entrance of the primary pulse-wave. This record was obtained from a typhoid fever patient who, on other occasions, showed a typical dicrotic pulse. Nitroglycerin will produce in some individuals a monocrotic pulse, in others a dicrotic pulse. In still others the typical monocrotic flow pulse present at the height of the action later becomes a dicrotic pulse as the effect of the

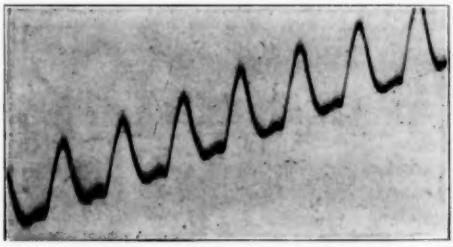

Fig. 3.—Marked monocrotic pulse.

drug wears off, an observation analogous to that of von Kries on the effect of amyl nitrite on radial tracings. It is, I believe, quite certain that the typical dicrotic pulse and the typical monocrotic pulse (as above defined) are but subvarieties of a more general type in which the primary pulse-wave has a pointed form on account of the marked fall in the pressure tracing just after its entrance. This fall is accompanied by the exit of a considerable amount of blood from the larger arteries of the arm owing to a local reflection of the primary pulse-wave.

In a previous paper[3] it was shown that nitroglycerin produces a marked backward movement of the blood in the brachial artery just after the entrance of the primary pulse-wave, and my collaborators and I have endeavored to explain this backward movement solely from the point of view of the changed conditions in the local vascular

3. Hewlett, A. W.: Van Zwaluwenburg, J. G., and Agnew, J. H.: The Pulse Flow in the Brachial Artery, THE ARCHIVES INT. MED., 1913, xii, 13.

apparatus of the arm. It was assumed that a relaxed condition of the larger vessels of the arm allowed an unusually large primary wave to enter this region, and that the reflection of this wave by the relatively constricted finer arterioles was a sufficient explanation of the backward movement of blood in the brachial artery just after the entrance of the primary wave. An attempt to apply this explanation to the dicrotic pulse of fever encounters certain difficulties. In the accompanying table I have given the records from our seven febrile patients who presented definitely palpable dicrotic pulses. In addition to the brachial flow records, which give the average rate of blood-flow in the arm and the height of the primary wave above this average flow, I have determined the velocity with which the pulse wave moved down the arm, by making simultaneous pressure tracings of the radial and of the carotid or brachial pulse-curves. The blood-pressure records in the table were in most but not in all instances taken at the same time as the other records.

PALPABLE DICROTIC PULSE

Patient	Diagnosis	Rate of Blood Flow per 100 c.c. Arm Substance per Minute, c.c.	Height of Primary Wave Above Average Inflow, c.c.	Velocity of Pulse Wave, cm.	Blood-Pressure	
					Max.	Min.
Mor.	Typhoid.....	2.5	0.5	840	120	76
Bus.	Typhoid.....	2.5-4	0.35-0.5	650	112	
V. H.	Typhoid.....	2.2	0.8	650	115	90
Barn.	Tuberculosis	2.5	0.45	750	130	
Mered.	Tuberculosis	2.0	.....	...	110	72
O'L.	Tuberculosis	2.5	0.6	830	95	
Bo.	Pneumonia..	1.7	0.4	870	108	57

It will be seen from this table that in all of the cases studied the average rates of blood-flow in the arm during marked dicrotism approached the lower level of the normal, which, by the method used, usually lies between 2 and 8 c.c. of blood per hundred c.c. of arm substance per minute. It is evident, therefore, that in cases of marked dicrotism there is no increased blood-flow in the arm, and consequently no marked dilatation of the finer arterioles in this vascular area. It cannot, of course, be inferred that there is likewise no vasodilatation of the arterioles in other important vascular areas and especially in the splanchnic region, for we know that an antagonism frequently exists between these vessels and those of the extremities.

It is likewise apparent from the table that the size of the primary pulse wave in the exquisite type of dicrotic pulse here studied is if anything less than the average. Thus in a series of twenty-five individuals showing various clinical conditions, exclusive of aortic insufficiency, the size of the primary pulse-wave as measured above the average rate of inflow varied from 0.4 to 1.1 c.c. with an average of

0.63 c.c.; while in our dicrotic pulses the primary wave varied from 0.35 to 0.8 c.c., with an average of 0.51 c.c. In this respect, therefore, the brachial flow in the dicrotic pulse differs from that present after the administration of nitroglycerin, for in the latter the size of the primary wave is almost invariably considerably larger than what was normal for the individual studied. It is thus evident that in the dicrotic pulse of fever with its close resemblance to the nitroglycerin pulse we lack one of the features which led us to attribute the latter chiefly to changes in the local blood-vessels of the arm itself. In the dicrotic pulse the primary wave which enters the arm is not unusually large.

The condition of the arteries of the arm is not easily determined, but from palpation one gets the impression that in the dicrotic pulse of fever the size of these vessels does not differ greatly from the normal. Furthermore, the rate of propagation of the pulse-wave is not markedly or constantly changed from the normal, which varies from about 670 to 1,000 cm. per second, with an average of about 825 cm.[4] Since the rate of propagation depends largely on the elastic coefficient of the arterial walls, it would appear that a marked reduction in the elastic coefficient of the larger vessels of the arm is not a constant and essential factor in the production of the dicrotic pulse of fever.

Our records indicate, therefore, that marked instances of the febrile dicrotic pulse are associated with moderate to marked constriction of the finer arterioles in the arm and with the entrance into the arm of primary waves of normal or subnormal size. So far as we can judge, there are no marked and constant changes in the condition of the larger arteries of the arm. It seems difficult to explain the marked oscillations in the brachial column of blood from these data alone and we are inclined to refer the dicrotic pulse of fever, in part at least, to cardiovascular changes outside of the arm. Before discussing the character of these changes, however, we wish to bring further evidence in favor of the view that a reflection of the primary pulse-wave in the arm is an important factor in the production of the pointed primary wave, which is such a prominent feature of dicrotic and monocrotic pulse-forms.

RELATION OF POINTED PULSE-FORMS TO REFLECTIONS OF THE PRIMARY PULSE-WAVE IN THE ARM

The view that dicrotism is associated with marked reflection of the primary pulse-wave in the arm stands in apparent contradiction to the commonly accepted view that dicrotism is most frequently encountered in conditions of vascular relaxation. According to this view, as

4. Friberger, R.: Ueber die Pulswellengeschwindigkeit bei Arterien mit fühlbarer Wandverdickung, Deutsch. Arch. f. klin. Med., 1912, cvii, 280.

described for example by Hirschfelder, the flow of blood through the arterioles is so rapid that a very marked fall of arterial pressure occurs even during the systolic portion of the pulse. Our records show that so far as the arm is concerned no such rapid flow of blood is present.

Indeed, if the arterioles of the arm are markedly dilated and the local blood-flow is very fast, the pulse tends to lose its dicrotic character. Very rapid local flows in the arm can be produced by having an

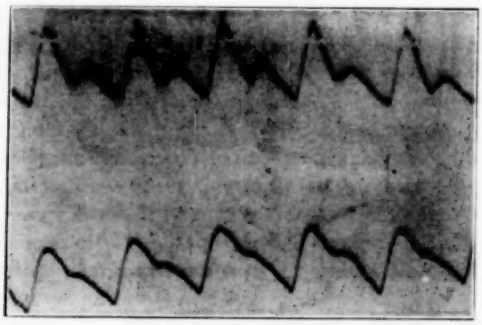

Fig. 4.—Volume pulse of the arm. Effect of constricting the arteries at the wrist after taking a very hot bath. Lower tracing without constriction, upper with constriction.

Fig. 5.—Volume pulse of the arm. Effect of constricting the arteries at the wrist after taking a very hot bath. Lower line without constriction, upper with constriction.

individual remain in a tub of very hot water until free perspiration is established and the body temperature is slightly elevated. The volume pulse then assumes the form shown in the lower curves of Figures 4 and 5. It is characterized by a gradual fall in the tracing and by a dicrotic wave which occurs relatively high on the tracing, both being characteristics opposite to those which are present in typical dicrotism.

Veiel[5] observed similar changes in the radial pressure pulse after a hot bath.

According to Stewart,[6] a compression of the radial pulse below the sphygmograph will cause the typical pulse of aortic insufficiency to lose its collapsing character. According to Marey (Fig. 138), on the other hand, such a compression may increase the dicrotism of a normal pulse. It is well known that sphygmographic tracings are liable to be influenced by gross technical errors, and we have therefore sought to make similar observations on the volume pulse of the arm when the arteries about the wrist were compressed. This was accomplished by placing a pressure cuff about the wrist within the plethysmograph and applying pressures of 200 mm. or more of mercury. The rubber tube to the pressure cuff was carried out of the plethysmograph, and it was therefore possible to obtain alternate tracings of the volume pulse or of the brachial flow when the wrist arteries were compressed and when they were left open.

The effect of obstructing the arteries at the wrist varied in different individuals and in the same individual at different times. In many cases the compression of the wrist produced little effect on the form of the volume curve; but in no case have we encountered any marked tendency toward diminished dicrotism as a result of such pressure. On the other hand, a number of records were obtained in which an increased dicrotism appeared when the wrist arteries were compressed. This effect was obtained with considerable regularity when the very rapid blood-flow after a hot bath was obstructed at the wrist by a pressure cuff (Figs. 4 and 5). One of our typhoid fever patients who had shown a typical dicrotic pulse on several occasions showed on one examination a rapid blood-flow through the arm (10 c.c. per hundred c.c. of arm substance per minute) and a pulse that was not definitely dicrotic. Compression of the wrist arteries slowed the blood-flow to 3 c.c., and at the same time the pulse took on a dicrotic character (Fig. 6).

Since the pressure curves from arteries in the arm follow nearly the same course as do the volume curves, it seems probable that similar changes in the pressure curves were present. These observations furnish further evidence in support of the view that one of the important factors favoring the production of a sharp fall in the volume (and pressure) tracings immediately after the primary wave

5. Veiel, E.: Ueber die Bedeutung der Pulsform, Deutsch. Arch. f. klin. Med., 1912, v, 249.

6. Stewart, H. A.: Experimental and Clinical Investigation of the Pulse and Pressure Changes in Aortic Insufficiency, THE ARCHIVES INT. MED., 1908, i, 102.

is a constriction of the finer arterioles of the arm which tends to cause a reflection of the primary wave in this region.

THEORIES OF THE POINTED CHARACTER OF THE PRIMARY WAVE IN DICROTIC AND MONOCROTIC PULSE FORMS

We have seen that the common and probably the most important feature of the dicrotic and monocrotic pulse-forms is the marked backward movement of blood in the brachial artery just after the primary pulse-wave, which movement gives rise to a pointed form of this wave in both volume and pressure records. Although present data hardly permit a satisfactory explanation of this pointed type of pulse, it may be of interest to review certain theories in the light of our observations and to suggest possible explanations for this type of pulse. I have shown previously that in the nitroglycerin pulse the marked backward movement of blood in the brachial artery during late systole could not be attributed solely to a settling back of the blood-

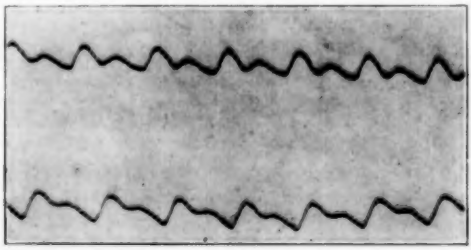

Fig. 6.—Volume pulse of the arm. Effect of constricting the arteries at the wrist in a patient with typhoid fever. The lower curve without constriction. Blood flow, 10 c.c. The upper tracing with constriction. Flow reduced to about 3 c.c.

column against the aortic valves. In that type of pulse as well as in those discussed in the present paper, the movement backward in the brachial artery is altogether too great to be accounted for in this manner. The only explanation which seems possible for this backward movement is that it is due to a reflection of the primary wave in the arm. New evidence in favor of this view is given in the present paper, and it is supported by a comparison of volume curves from the arm and from the hand, which are published elsewhere.[7]

In the nitroglycerin pulse an unusually large primary wave enters the arm, and in a previous paper I sought to explain that type of pulse solely on the basis of changes in the arm vessels. In the dicrotic pulse

7. Hewlett, A. W.: Reflexionen der primären Pulswelle im menschlichen Arme, Deutsch. Arch. f. klin. Med., 1914, cxvi, 237.

of fever, however, such an explanation encounters the difficulty that the primary wave may be unusually small and yet the backflow in the brachial artery may be relatively large. For this and other reasons it does not seem possible to account for the peculiar features of the dicrotic pulse solely on the basis of circulatory changes within the arm. It seems to us necessary to assume that changes in the cardiac or vascular mechanism outside of the arm play an important part both in the production of the backward movement of late systole and in the size and time of onset of the dicrotic wave.

Just what these conditions are is difficult to determine. Since the primary wave entering the arm interferes to a greater or lesser extent with its own reflection, a separation of these two in point of time would tend to make the reflected wave more evident on our tracings. Such a separation might be produced by a relatively slow rate of propagation of the pulse wave in the arm; but this, as we have seen, does not appear to be a constant finding in the types of pulse here studied. A short primary wave due to a rapid systolic emptying of the heart would also diminish this interference, and in cases of tachycardia as well as certain extrasystoles the frequently observed pointed character of the pulse may be due to this cause. Whether or not this factor plays a part in the backflows here described we do not know.

The known facts in regard to these pulse-forms may also be explained in accordance with the views of von Kries. This author concluded from theoretical studies on wave motion in elastic tubes and from comparisons of the pressure pulse and the tachogram of the arm of man that reflections of the primary pulse waves usually take place in the arm. My tracings show that under certain conditions such reflections produce well-marked backflows just after the entrance of the primary pulse wave. It is highly probable that similar reflections occur at numerous other points in the vascular tree and that these complicated systems of reflected waves return to the aorta and may then be propagated as centrifugal waves into various arteries. Under normal conditions these numerous and complicated systems of reflected waves tend to neutralize one another by interference, and the pulse approaches the type produced by the intermittent expulsion of fluid into an elastic bag with a narrow outlet. Von Kries explained the changes in pulse form produced by amyl nitrite by assuming that, owing to vascular dilatations in parts of the body other than the arm, certain of these reflected waves were absent and that the absence of these reflected waves which would otherwise have entered the arm between the primary and the dicrotic waves was the cause of the marked fall in pressure after the primary wave.

A similar explanation may be given for the pointed character of the primary wave in the dicrotic and monocrotic pulse-forms of fever, and it may well play an important part in the production of other pointed pulse-forms. A vasodilatation of certain vascular areas outside of the arm may diminish or eliminate certain reflected waves which would otherwise enter the arm between the primary and the dicrotic waves. This would allow the reflection of the primary wave in the arm to become apparent on our tracings as a negative blood-flow in the bracial artery. Knowing the rate of propagation of waves in the arterial system it is possible to estimate the distance which must be traveled in order that waves reflected from other parts of the vascular apparatus should enter the arm shortly after the primary wave. Such a calculation shows that these waves would be reflected from points lying in the neighborhood of 35 to 60 cm. beyond the points of origin of the subclavian arteries. The most important vascular areas lying at about this distance are the abdominal vessels. It is possible, therefore, that the pointed form of the primary wave in the dicrotic and monocrotic pulse of fever may be due in part at least to an unusual dilatation of the splanchnic vessels. This hypothesis is in accord with many statements concerning the conditions which produce these pulse-forms. Thus Cushny[8] states that amyl nitrite produces a more marked dilatation of the vessels in the head and the abdomen than in those of the extremities. Fever is likewise generally supposed to cause a dilatation of the splanchnic vessels. The frequent but not invariable association between low blood-pressure and dicrotism may be due to the fact that while dilatation of the splanchnic vessels frequently lowers the blood-pressure it may fail to do so owing to the constriction of vessels in other parts of the body. A constriction of the arterioles in the arm would tend to maintain the blood-pressure and at the same time it would increase the local reflection, provided the larger arteries of the arm were not correspondingly constricted.[3]

CONCLUSIONS

1. In febrile patients showing palpable types of the dicrotic pulse, the brachial flow curves show characteristic changes. These consist in a marked backflow in the brachial artery just after the entrance of the primary pulse-wave and in an inflow and outflow with the dicrotic wave.

2. In the form of pulse here described as monocrotic there is also a marked outflow just after the primary pulse-wave, but no marked dicrotic wave follows. This type of pulse is closely related to the

8. Cushny, A. R.: Pharmacology and Therapeutics, 1906, p. 465.

typical dicrotic pulse. The primary wave in each has a pointed character.

3. Other things being equal, the pointed character of the pulse in the arm is increased by vasoconstriction of the arm arterioles and is diminished by their relaxation.

4. We have been unable to explain all forms of the pointed pulse satisfactorily solely on the basis of local vascular changes in the arm; that is, constriction of the arterioles and relaxation of the larger arteries. In many, if not in all cases, factors outside of the arm play a part in their production.

5. Among such possible factors are (1) a brief systolic output from the heart and (2) a dilatation of certain vascular areas outside of the arm, the most important being the splanchnic vessels.

THE TOTAL NON-PROTEIN NITROGEN AND THE UREA OF THE BLOOD IN HEALTH AND IN DISEASE, AS ESTIMATED BY FOLIN'S METHODS*

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Ever since the time Prévost and Dumas,[1] who in 1823, first demonstrated an increase of the urea of the blood after extirpation of the kidneys in animals, the total non-protein nitrogen and the urea of the blood have been the subject of repeated investigations, and have been accorded considerable importance in the diagnosis and prognosis of Bright's disease. Owing, however, to the fact that the methods employed have been various and more or less subject to error, the results obtained have been conflicting. This may be readily seen when we find that the total non-protein nitrogen in the normal person is given as anywhere from 25 to 60 mg. per hundred c.c. of blood. The brilliant methods recently devised by Folin[2] render possible the accurate estimation of these substances in a small amount of blood, from 2 to 5 c.c. sufficing for all the analyses. The increase in accuracy depends on an improved method of removing the proteins from the blood, and the use of Nessler's solution makes it possible to work with small quantities of blood.

The term "non-protein nitrogen" explains itself. It includes all the nitrogenous substances remaining after the removal of the proteins by precipitation, in the case of Folin's method by means of methyl alcohol and later by zinc chlorid. Other names for it are "incoagulable nitrogen," "filtrate nitrogen," "rest," "waste" or "retention nitrogen."

Folin's method for total non-protein nitrogen is essentially a "micro-Kjeldahl" process, in which the ammonia after neutralization of the products of digestion is blown over into a collecting vessel by a current of air instead of by distillation, and is estimated by the use of Nessler's solution and the colorimeter as in water analysis. His method for urea depends on the quantitative breaking down of this substance to ammonia at a temperature of 150 C., and its subsequent estimation in the same manner as the total nitrogen. The figures

* Submitted for publication Aug. 11, 1914.
* From the Department of Medicine, Yale University Medical School.
* Read in abstract at the meeting of the Association of American Physicians, May 13, 1914.

1. Prévost and Dumas: Ann. de chimie et de physique, 1823, xxiii, 90.
2. Folin, O., and Denis, W.: Jour. Biol. Chem., 1912, xi, 527.

obtained represent urea estimated as nitrogen, and include the ammonia nitrogen, which, however, is so small in normal blood, and presumably in most forms of disease, as to be negligible.

These new methods of Folin open up great possibilities both in the line of experimental research and in clinical work, and promise to throw new light on many problems connected with the function of the kidneys.

The present investigation was undertaken with the view of determining the non-protein nitrogen and the urea of the blood in a large series of miscellaneous diseases, paying special attention of course to affections of the kidneys. In this way it was hoped to gain a more exact idea as to the diagnostic and prognostic value of such determinations. The figures of previous investigators along these lines, of whom Strauss,[3] Hohlweg[4] and Widal[5] are especially deserving of mention, are not comparable to those obtained by Folin's methods, for reasons already given. The work of the French school is especially inaccurate, depending on the very faulty hypobromite method for the urea estimation, a method which Rowntree and Fitz[6] have shown to involve an error of from 10 to 60 per cent. We shall consider the results obtained by others working with the new Folin methods later on.

Before passing to a detailed account of our work, however, we cannot refrain from alluding to a practice of which H. Strauss has been guilty. This author, to whom we owe so much in the investigation of Bright's disease, has published as his own[7] a slight modification of Folin's method for total nitrogen, giving Folin credit only for devising the method of removing the albumin. His method is word for word the same as Folin's up to the stage of combustion, where he substitutes an ordinary Kjeldahl determination on a large amount of filtrate for Folin's "micro-Kjeldahl" process. His offense is rendered more flagrant by the fact that he went to Folin's laboratory to learn Folin's method before publishing his article.

TECHNIC

We have followed very closely the original technic as described by Folin and Denis,[2] and learned by one of us (Tileston) in Professor Folin's laboratory. The material has consisted of whole blood, oxa-

3. Strauss, H.: Die chronische Nierenentzündungen in ihrer Einwirkung auf die Blutflüssigkeit, Berlin, 1902; also Deutsch. Arch. f. klin. Med., 1912, cvi, 219.

4. Hohlweg, H.: Deutsch. Arch. f. klin. Med., 1911, civ, 216.

5. Widal, F.: Bull. et mém. Soc. méd. d. hôp. de Paris, 1911, xxxii, 627. (And many other articles.)

6. Rowntree, L. G., and Fitz, R.: The Archives Int. Med., 1913, xi, 258.

7. Strauss, H.: Ztschr. f. Urol., 1913, vii, 287.

lated, and the results have been calculated as milligrams of nitrogen per 100 c.c. of blood. In order to avoid the effects of the ingestion of protein, the blood has been drawn in the forenoon when possible. In cases with very large amounts of nitrogen we have found it advantageous to take 2 c.c. instead of 5 c.c. of the filtrate for analysis, and to dilute the specimens with water before adding Nessler's solution, thus avoiding turbidity. The determinations have been made in duplicate, with further controls in case the figures did not agree closely. The methods have proved accurate and reliable in our hands, and for any one with an eye for color, are not much more difficult to learn than the ordinary Kjeldahl method.

THE TOTAL NON-PROTEIN NITROGEN AND THE UREA IN HEALTH

Folin and Denis,[8] working with the new methods, found in a series of sixteen healthy adults the total non-protein nitrogen varying within narrow limits, from 22 to 26 mg. per 100 c.c. of blood, while the urea nitrogen was exactly half as much, from 11 to 13 mg. The blood was taken in the forenoon, from three to six hours after breakfast. The figures given by previous investigators, as already mentioned, are all too high by reason of faulty methods.

TABLE 1.—NORMAL *

Case	Age	Total Nitrogen (mg.)		Urea Nitrogen (mg.)	
		After fasting 12 hrs.	2½ hrs. after heavy meal	After fasting 12 hrs.	2½ hrs. after heavy meal
D. G. R.	24	22.9	25.9	12.0	13.6
L. V. G.	26	25.0	27.9	13.6	14.5
E. M. J.	27	23.7	29.5	12.6	15.1
C. W. C.	29	23.8	32.3	14.1	20.9
W. T.	39	23.2	26.4	12.3	13.1

* In this and the following tables, the figures for total nitrogen and for urea nitrogen represent milligrams per 100 c.c. of blood.

In order to study the influence of diet, we examined five healthy male adults, early in the morning after a twelve-hours fast, and again in the afternoon of the same day, two and one-half hours after a meal containing a large amount of meat. The urine was normal in all these persons. As will be seen by a glance at Table 1, our fasting figures agree very closely with those of Folin, the nitrogen varying from 23 to 25 mg., and the urea from 12 to 14 mg. The effect of a heavy protein meal is seen to be a rise in the non-protein nitrogen of from 2.9 to 8.5 mg., averaging 4.7 mg., and a rise in the urea averaging

8. Folin and Denis: Jour. Biol. Chemistry, 1913, xiv, 33.

2.5 mg. One of our subjects (C. W. C.), who ate a great deal of meat, shows such a divergence from the others that an abnormality of the renal function seems possible, though the urine was negative. Not only did the nitrogen rise to a high figure after the meal (32 mg.), but the urea amounted to considerably more than one-half the total nitrogen (65 per cent.).

The results show the need of considering the ingestion of food in interpreting the results of blood analysis, and the advisability of taking the blood either fasting or before the chief meal of the day. Further studies along these lines are desirable.

THE TOTAL NON-PROTEIN NITROGEN AND THE UREA IN DISEASE

One hundred and forty-two cases of disease of one sort or another have been studied. For the purpose of classification they have been divided into eleven groups, as follows: (1) chronic nephritis; (2) other diseases of the kidneys and of the genito-urinary tract; (3) lead-poisoning; (4) the complications of pregnancy; (5) acute intestinal obstruction; (6) diseases of the heart and aorta; (7) the acute infections; (8) syphilis; (9) tuberculosis; (10) diseases of the nervous system; (11) miscellaneous diseases. These will be taken up in order.

For practical purposes figures for nitrogen below 30 mg. may be considered normal; those from 30 to 35 slightly, from 35 to 50 considerably, and from 50 to 100 greatly increased. One hundred milligrams or more, as we shall see later, constitutes a very dangerous elevation of the waste nitrogen. In the case of urea nitrogen, anything over 16 mg. is probably abnormal, and above 25 mg. considerably increased.

In the tables the figures for total nitrogen and urea nitrogen represent milligrams per hundred c.c. of blood. The abbreviations are explained under Table 2.

I. CHRONIC NEPHRITIS

A. *Chronic Interstitial Nephritis.*—In this disease we find figures varying all the way from normal to the enormous amount of 324 mg. for the nitrogen and 237 mg. for the urea. The seventeen cases fall naturally into two groups, those with normal or slightly elevated figures, and those with a marked increase. In the former, with one exception (Case 7), we have found a complete absence of all symptoms referable to renal insufficiency, although the hypertension in some was of long standing. These patients were mostly of the elderly, arteriosclerotic type. In the group with high nitrogen and urea, more or less pronounced symptoms of uremia have been present in all, excepting the border-line case, No. 12, with 57 mg. nitrogen. Of the six

patients with over 100 mg. of nitrogen, five died within a period of 35 days, the sixth (Case 13) who came into the hospital with well-marked symptoms of chronic uremia, and 80 mg. of nitrogen, became worse at first, and the retention nitrogen rose to 102 mg.; then improvement set in, and he left the hospital in fair condition at the end of two months, with a blood nitrogen of 70 mg. But the improvement was only temporary. He has just entered the hospital again with the old symptoms, and a blood nitrogen of 88 mg. fourteen weeks after the first examination.† Farr and Austin[9] report a still more striking case (No. 31 of their series) of uremia with delirum tremens in which the nitrogen fell from 181 mg. to 36 mg. under treatment, and the patient was discharged in good shape. The subsequent history of these patients would be interesting. Javal[10] states that such patients usually relapse and die within a few months, and reports[11] one patient surviving eight months and still alive, as extremely exceptional.

We have encountered no case of uremia of the asthenic type, that is, with weakness and apathy, and gastro-intestinal symptoms—which has not shown a very marked increase of retention nitrogen. The great importance of blood analysis in the diagnosis of uremia, and in the prognosis of chronic nephritis, is evident from these facts.

The single case of uremia without retention of nitrogen (Case 7) calls for a more detailed description. The patient, a woman 64 years old, entered the hospital in a stuporous condition, a few hours after an epileptiform attack. The urine was consistent with the diagnosis of chronic nephritis; the systolic blood-pressure was 205 mm. and fell to 185 after venesection. No more convulsions occurred and the next day the patient was better and clear mentally. Eleven days later she was discharged in good condition. There was a history of an attack of unconsciousness, probably without convulsions, ten months previously. In the absence of evidence pointing to epilepsy, alcoholism or syphilis, it seems reasonable to attribute the convulsion to nephritis. The non-protein nitrogen was 31.5, the urea 16.4 on the day of entrance; the phthalein output was 42 per cent. There was evidently no renal insufficiency. This case fits in with the epileptiform type of uremia described recently by Reiss,[12] in which there are convulsions exactly resembling those of epilepsy, with euphoria in the intervals, no increase in the non-protein nitrogen of the blood and no deficiency

† Note on reading proof: This patient died Aug. 11, 1914, of uremia, eighty-eight days after the nitrogen reached 100 mg.

9. Farr, C. B., and Austin, J. H.: Jour. Exper. Med., 1913, xviii, 228.
10. Javal, A.: Bull. et Mém. Soc. méd. d. hôp. de Paris, 1911, xxxii, 485.
11. Mosny, Javal and Levy-Bruhl: Bull. et mém. Soc. méd. d. hôp. de Paris, 1911, xxxii, 374.
12. Reiss, E.: Ztschr. f. klin. Med., 1914, lxxx, 97.

in renal function. Such cases have been described by other authors, and Strauss pertinently asks if they are to be considered as truly of uremic origin.

B. Chronic Diffuse Nephritis.—We have examined only two cases of this disease. The first (Case 18) showed anasarca at entrance, with large amounts of albumin in the urine. The total nitrogen was normal and the urea unusually low (35 per cent. of the total non-protein nitrogen); there were no uremic symptoms. The normal nitrogen figures frequently encountered in chronic diffuse nephritis have been ascribed to the hydremia or dilution of the blood. That this is not necessarily so was shown by later examination in this case after the edema had disappeared; the nitrogen was even lower than before. The second case (No. 19) entered the hospital with moderate edema, a normal blood-pressure and mild uremic symptoms. During his long stay the nitrogen gradually rose from 55 mg. to 67 mg. five weeks later, then rapidly to 174 mg. the day before his death from uremia, five months after the first examination. Meanwhile the blood-pressure rose to over 200 mm. and he developed a high grade of albuminuric retinitis. At the autopsy there was a marked chronic nephritis with hypertrophy of the heart (weight 600 gm.) and moderately advanced tuberculosis of the lungs. The combined weight of the kidneys was 300 gm. Microscopically, there was very extensive glomerular disease; the tubules were dilated and filled with casts, and here and there showed degeneration and desquamation of the epithelium; there was a dense infiltration of the interstitial tissue with lymphoid cells.

The single case of amyloidosis (No. 20) showed practically normal figures.

C. Acute Nephritis.—Of this disease we have but one example to report, a case of acute arsenical poisoning ending in recovery (Case 21, Table 3). Two days after taking a large dose of Paris green the nitrogen was 70 mg. and the urea 58 mg. With convalescence the figures sank to normal. There was no edema. This case is comparable to the experimental uranium nephritis investigated by Frothingham,[14] who found a marked increase of retention products at the height of the disease.

Before leaving the subject of Bright's disease we wish to discuss the relationship of albuminuric retinitis to retention. Widal[15] claims that this condition is always an evidence of azotemia, which is his

13. Strauss, H.: Deutsch. Arch. f. klin. Med., 1912, cvi, 219.

14. Frothingham, C., Fitz, R., Folin, Otto, and Denis, W.: The Relation Between Non-Protein Nitrogen Retention and Phenolsulphonephthalein Excretion in Experimental Uranium Nephritis, THE ARCHIVES INT. MED., 1913, xii, 245.

15. Widal, F.: Presse méd., 1912, xx, 973.

TABLE 2.—

Case No.	Sex	Age	Diagnosis	Systolic Blood-Pressure mm. Hg	Urine			
					Phenol-sulphone-phthalein % in 2 hrs.	Amt. c.c. in 24 hours	Sp. Gr.	Alb.
1	M	41	Chronic interstitial	180	66	1,300	1.017	S. P. T.
2	F	62	Chronic interstitial	195	..	600	1.023	S. P. T.
3	F	52	Chronic interstitial	210	40	(?) 390	1.019	S. P. T.
4	F	75	Chronic interstitial	210	38	460	1.019	S. P. T.
5	F	66	Chronic interstitial	220	38	S. S.	1.012	S. P. T.
6	M	65	Chronic interstitial	200	..	S. S.	1.030	S. P. T.
7	F	64	Chronic interstitial	205	42	1,050	1.020	V. S. T.
8	F	46	Chronic interstitial	180	50	520	1.019	V. S. T.
9	F	61	Chronic interstitial	250	..	S. S.	1.010	S. P. T.
10	F	63	Chronic interstitial, with albuminuric retinitis	185	..	2,040	1.018	V. S. T.
11	F	53	Chronic interstitial	240	..	2,000	1.007	S. P. T.
12	F	24	Chronic interstitial, with cholelithiasis	...	80	650	1.014	S. T.
13	M	48	Chronic interstitial, with uremia	240	22 .. 26	530	1.016	V. S. T.
14	M	25	Chronic interstitial with albuminuric retinitis	212	0.0	279+	1.011	0.8%
15	M	55	Chronic interstitial, with uremia	210	0.5	730	1.010	S. T.
16	M	22	Chronic interstitial, with uremia	180	0.5	1,030	1.014	S. T.
17	M	25	Chronic interstitial with uremia	175	0.0	S. S.	1.010	T.
18	F	20	Chronic diffuse	132	43	420	1.030	2.0%
19	M	25	Chronic diffuse	146	5	960	1.016	0.4%
				210	0.0	1,440	1.010	H. T.
20	M	44	Amyloidosis	...	55	S. S.	1.026	S. T.

* The following abbreviations are employed in this and the following tables: S. S. S. T. = slight trace, T. = trace, H. T. = heavy trace, W. B. C. = white blood-cor Esbach method, and represent percentages.

Urine		Blood		
Microscopical	Diet	Total N. (mg.)	Urea N. (mg.)	Remarks
Few hyaline casts; W. B. C.	Mixed	23.0	11.4	Early process in kidneys. Wassermann +. No other indications of syphilis.
Numerous hyaline casts: few R. B. C.	Mixed	23.2	11.1	Recent attack of cardiac decompensation. No sign of renal insufficiency.
Rare hyaline cast; few W. B. C.	Mixed	27.4	13.4	
Few hyaline, granular casts; W.B.C.	Mixed	28.2	14.6	No symptoms.
Pus	Moderate protein	29.0	16.1	Complicated with cystitis. Acute pyelitis several months ago. Cerebral hemorrhages 1 year and again 1 month ago.
	Poor protein, 1 day	25.3	....	
.....	Mixed	29.7	16.1	Entered for epistaxis. No sign of renal insufficiency.
Rare hyaline casts; W. B. C.	Mixed	31.5	16.4	One epileptiform convulsion a few hours previous. Prompt recovery after venesection, without any more fits.
Occasional coarse granular cast; many W. B. C.	Soft	31.7	15.4	Subacute appendicitis recently. Early process in kidney.
Very few hyaline casts; some R.B.C.	Mixed	31.7	16.7	Blood-pressure over 200 for past 4 years. Never any symptoms of uremia.
Occasional hyaline cast; many R.B.C.	Mixed	32.0	17.7	Albuminuric retinitis of moderate intensity, one year's duration.
Rare hyaline cast	Mixed	35.3	....	Hypertension for several years. No sign of renal insufficiency.
Hyaline, occasional granular casts; W. B. C.	Soft	57.5	27.7	Recent attack of gall-stone colic.
	Low protein, 6 days	24.2	12.5	No symptoms of uremia.
Hyaline, granular casts; R. B. C.; W. B. C.	Mixed	79.5	55.1	4/29/14 Lead poisoning probable. Symptoms of chronic uremia. Left hospital improved after 2 mos.
		102.2	79.5	5/15/14
		70.5	40.4	6/21/14
		87.5	53.1	8/ 8/14 Returned in 6 weeks in same condition as before.
Hyaline, granular casts; R. B. C.; W. B. C.	Milk	151.2	109.4	Probably secondary contracted kidney. Slight edema, headache, vomiting, blurred vision. Died 2 days later.
Hyaline, granular casts; many R. B. C.; W. B. C.	Mixed	188.7	141.0	4/23/14 Symptoms of chronic uremia at
		286.2	191.0	5/13/14 entrance. Died uremic 35 days after first analysis.
Few hyaline, granular casts; R.B.C. W. B. C.	Low protein, 5 da., mixed, 3 days	220.0	143.0	3/10/14 Entered with symptoms of
		232.5	184.0	3/13/14 chronic uremia. Second entrance
		324.0	237.0	3/27/14 3/27/14. Died in coma 2 days after last analysis. Necropsy: small granular kidneys, combined weight, 105 gm.
Few hyaline, granular, epithelial casts; W. B. C.	Liquid	313.0	205.0	Died next day in coma, preceded by convulsions.
Many hyaline, granular casts; R.B.C. W. B. C.	Soft	26.7	10.7	2/13/14 Considerable edema at first anal-
	Salt free, 5 weeks	22.6	8.0	3/19/14 ysis; none at second. Discharged improved, 4/29/14.
Many hyaline, granular casts; R.B.C. W. B. C.	Mixed	55.2	31.5	1/26/14 At first admission, occasional
		67.0	49.0	3/ 7/14 slight symptoms of uremia, head-
		140.6	113.1	5/16/14 ache, dizziness, nausea, vomiting.
		173.7	131.3	5/20/14 Moderate general edema. Tuberculosis of lungs, 2d stage. Second admission 5/9/14, with pronounced uremic symptoms and albuminuric retinitis. Died uremic, 5/21/14. Necropsy showed very extensive disease of glomeruli, tubules and interstitial tissue.
.....	Mixed	33.9	15.3	Enlarged liver and spleen; osteomyelitis with discharging sinuses for 1 year.

= single specimen, S. P. T. = slightest possible trace, V. S. T., = very slight trace; puscles, R. B. C. = red blood-corpuscles; the figu res for albumin were obtained by the

TABLE 3.—Other Diseases of—

Case No.	Sex	Age	Diagnosis	Systolic Blood-Pressure mm. Hg	Urine			
					Phenol-sulphone-phthalein % in 2 hrs.	Amt. c.c. in 24 hours	Sp. Gr.	Alb.
21	M	48	Acute nephritis	85	48	590	1.013	V. S. T.
22	M	58	Chronic passive congestion	128	70	450+	1.022	S. P. T.
23	M	54	Chronic passive congestion	170	49	850	1.024	V. S. T.
24	M	58	Chronic passive congestion	120	50	1,660	1.011	S. P. T.
25	M	45	Chronic passive congestion	90	58	620	1.024	V. S. T.
26	M	47	Chronic passive congestion	115	53	500	1.023	S. T.
27	M	21	Chronic passive congestion	110	75	1,680	1.017	S. P. T.
28	F	16	Chronic passive congestion	...	..	S. S.	1.040	H. T.
29	F	27	Pyelitis	118	80	1,900	1.004	S. P. T.
30	F	19	Pyelitis	112	30	1,340	1.015	V. S. T.
31	M	47	Hypernephroma	125	..	1,220	1.018	S. T.
32	M	62	Stricture of urethra, with acute ascending infect.	180	..	S. S.	1.030	H. T.
33	M	63	Stricture of urethra, with acute ascending infect.	...	..	S. S.	1.020	T.
34	M	69	Tuberculosis of genito-urinary tract	88	10	1,100	1.014	S. T.
35	M	86	Hypertrophied prostate with retention	85	..	.....	.....	.....
36	M	77	Hypertrophied prostate with retention	142	60	S. S.	1.022	T.
37	M	64	Carcinoma, prostate and bladder	110	10	1,180	1.017	S. T.
38	M	70	Carcinoma, bladder	...	0.0	.....	.....	.....

—KIDNEYS AND GENITO-URINARY TRACT

Urine		Blood		
Microscopical	Diet	Total N. (mg.)	Urea N. (mg.)	Remarks
Hyaline, granular, pus casts; R. B. C. W. B. C.	Soft	69.7 29.5	57.7 15.7	4/25/14 Large dose of Paris green taken 5/18/14 2 days previously. Recovered.
Occasional hyaline cast; W. B. C.	Karell	24.6	13.7	Mitral regurgitation; cardiac decompensation; marked edema.
Hyaline, granular casts; R. B. C.; W. B. C.	Karell	25.0	13.8	Aortic regurgitation; luetic aortitis.
Hyaline casts; W. B. C.	Soft	30.6	16.9	Mitral regurgitation and chronic cardiac decompensation.
Few hyaline, granular casts; W. B. C.	Mixed	31.7	15.1	Mitral stenosis; failure of compensation without edema.
Many hyaline casts; R. B. C.; W. B. C	Karell	34.4	17.5	Edema; extreme cyanosis; died 2 days later.
Hyaline casts; few R. B. C.; W. B. C.	Soft	42.2	25.4	Mitral stenosis and regurgitation with decompensation.
Many hyaline, granular casts; W.B.C.	Soft	67.7	43.9	Blood taken a few hours before death from failure of compensation, secondary to mitral disease.
Occasional hyaline cast; pus	Soft	26.5	14.9	Acute febrile attack.
Rare hyaline cast; R. B. C.; pus	Liquid, soft	70.7 40.2	51.0 29.2	6/16/14 Complicated with pyelonephrosis 6/26/14 at entrance.
Many R. B. C.; W. B. C.	Mixed	33.5	18.2	Tumor involving nearly all of left kidney. Removed later at operation.
Much pus	Liquid	91.2	61.2	Died next day. Necropsy: extravasation of urine; gangrenous cystitis with perforation of bladder; purulent pyelitis.
Many R. B. C. and W. B. C.	Soft	209.0	172.0	Hemorrhagic cystitis with putrid urine. Died same day of asthenia; no convulsions.
R. B. C.; very many W. B. C.	Soft, with meat once daily	31.4	19.1	Entered hospital for retention. Tubercle bacilli in urine. Location of disease uncertain. Died 8 days after.
.....	Liquid	50.0	37.6	Prostatectomy next day. Died 5 days later.
R. B. C.; W. B. C.	Mixed Low protein	48.7 30.5	37.6 17.2	3/13/14 Prostatectomy 5 days after sec- 4/ 1/14 ond analysis. Recovered.
R. B. C.; pus.....	Mixed	41.0	24.3	Suprapubic drain in bladder before blood examination.
.....	Soft	58.2 99.5	45.5 69.7	4/10/14 Operation on second day after 4/15/14 second analysis. Bladder filled with growth. Died 4 days later.

appropriate name for a marked increase of the nitrogenous waste products. That this is not always so is shown by our Case 10, in which a moderate grade of albuminuric retinitis of a year's duration was accompanied by normal figures for nitrogen and urea. Where the eye condition was advanced, however, we too have found marked azotemia.

II. OTHER DISEASES OF THE KIDNEYS AND OF THE GENITO-URINARY TRACT

A. Chronic Passive Hyperemia of the Kidneys.—In this condition we have found either normal or moderately elevated figures, even in cases with extreme congestion (cf. Case 26). The one exception was a

TABLE 4.—

Case No.	Sex	Age	Diagnosis	Systolic Blood-Pressure mm. Hg	Urine			
					Phenol-sulphone-phthalein % in 2 hrs.	Amt. c.c. in 24 hours	Sp. Gr.	Alb.
39	M	27	Lead colic	108	..	650	1.025	T.
40	M	26	Lead colic	160	..	675	1.030	S. P. T.
142	M	31	Lead colic	137	51	950	1.021	S. P. T.
41	M	35	Lead palsy	153	50	1,360	1.018	V. S. T.
42	M	36	Lead colic	188	..	620	1.022	0.05%
43	M	34	Lead colic	...	..	S. S.	.....	S. P. T.
44	M	63	Chronic plumbism, with chronic interstitial nephritis and uremia	205	..	S. S.	1.020	0.2%

young girl with very marked failure of compensation; the blood was taken only a few hours before death, and showed 70 mg. of nitrogen. No autopsy was permitted. So far as can be judged from a small series of seven cases, the absence of retention is the rule in pure passive hyperemia, and its presence would indicate a complicating nephritis or possibly infarction.

B. Pyelitis.—In one of the two cases examined there was a considerable retention, amounting to 71 mg. of nitrogen. This case was complicated with pyonephrosis; the retention almost disappeared during convalescence. The other case, examined during the febrile stage

showed normal figures. It seem probable that pyelitis in the absence of marked involvement of the kidney proper is not accompanied by azotemia.

C. Stricture of the Urethra, with Ascending Infection.—Two cases were encountered in this group. Both showed marked retention. The first patient (No. 32) suffered from extravasation of urine, the result of gangrenous cystitis with perforation of the bladder. The second (No. 33), a man with putrid hemorrhagic cystitis, the result of stricture, died with symptoms of uremia of the asthenic type. The blood nitrogen was excessively increased, measuring 209 mg.

D. Enlargement of the Prostate with Retention.—Two cases of simple hypertrophy of the prostate with retention of urine showed a

—Lead Poisoning

Urine		Blood		
Microscopical	Diet	Total N. (mg.)	Urea N. (mg.)	Remarks
Few hyaline, granular casts; R.B.C.; W. B. C.	Liquid	35.5	25.1	First attack.
Many hyaline casts; W. B. C.	Liquid	38.4	29.2	Attacks 2 years and again 1 week ago.
Occasional hyaline; rare R. B. C. W. B. C.	Mixed	42.2	23.4	Colic off and on for 2 years.
Few hyaline casts; W. B. C.	Soft	47.7	32.5	Drop wrist. Similar attack 3 years ago.
Many hyaline, granular casts; R. B. C.; W. B. C.	Soft	51.5	29.2	First attack. Exposed to lead many years.
Hyaline, granular casts	Liquid	54.0	....	First attack 1 year ago.
.....	Liquid	99.0	71.6	Painter. Colic 20 years ago and last year. Delirium and uremic coma. Autopsy: small granular kidneys.

considerable increase of the blood nitrogen, up to 50 mg. One patient (No. 35) was operated on immediately and died five days later. The other was put on a low protein diet for five days, at the end of which time the nitrogen had fallen to normal. Prostatectomy was then performed and the patient made a good recovery. In a case of cancer of the bladder (Case 38) in which the nitrogen rose in five days from 58 to 99 mg., the patient was operated on soon after the second blood examination and died four days afterward. The cases are too few to draw conclusions from, but it seems probable that patients with a high blood nitrogen are poor operative risks and that better results will be

attained by reducing the nitrogen by means of a diet poor in protein before operation.

III. LEAD-POISONING

The deleterious action of lead on the kidneys is clearly shown by the figures in Table 4. In every case there is a definite increase in nitrogen and urea, even in those entering hospital with their first attack of colic. All degrees of retention of waste products are seen, up to uremia (Case 44), the final event in so many cases of lead-poisoning.

TABLE 5.—COMPLICATIONS—

Case No.	Age	Diagnosis	Duration of Pregnancy Mos.	Systolic Blood-Pressure mm. Hg	Urine			
					Phenolsulphonephthalein % in 2 hrs.	Amt. c.c. in 24 hours	Sp. Gr.	Alb.
45	35	Eclampsia	6	128	18	340+	1.028	V. S. T.
46	21	Eclampsia*.....		118	65	680	1.020	V. S. T.
47	26	Eclampsia	7	220	30	S. S.	1.028	S. T.
48	28	Eclampsia	9	...	..	S. S.	1.024	H. T.
49	35	Eclampsia, threatened.	4	124	25	420	1.023	S. T.
50	22	Nephritis of pregnancy	6	151	..	620	1.030	0.5%
51	25	Nephritis of pregnancy	7½	170	.. 59	350	1.032	1.2%
52	29	Hyperemesis gravidarum	4	145	36	385	1.027	V. S. T.
53	30	Hyperemesis gravidarum	4	...	..	S. S.	1.022	T.

* Postpartum.

IV. THE COMPLICATIONS OF PREGNANCY

Farr and Williams,[16] working with Folin's methods, have shown that the figures in normal pregnancy are practically the same as in the non-pregnant.

The eclampsia of pregnancy has often been compared with uremia, and even considered identical with it. The results of blood analysis, however, show that in a large proportion of the cases there is little or no retention of nitrogenous waste products in eclampsia, while in uremia there is almost invariably a very marked retention. Thus all

16. Farr, C. B., and Williams, P. F.: Am. Jour. Med. Sc., 1914, cxlvii, 556.

of our four cases (Table 5) yielded figures for nitrogen below 40 mg., while six out of the seven cases of Farr and Williams were below 55 mg.; the seventh had 72 mg. In uremia we have found figures of 100 mg. or over in all but one of our cases.

This striking difference between the two conditions is of importance from the point of view of pathogenesis, indicating that eclampsia and uremia are quite distinct from one another. It is also of great

—OF PREGNANCY

Urine		Blood		
Microscopical	Diet	Total N. (mg.)	Urea N. (mg.)	Remarks
Many hyaline, granular casts	Milk	25.0	14.1	20 convulsions in last 48 hours. Developed acute mania. Recovered. Did not miscarry.
Few hyaline casts	Liquid	29.5	18.5	Three convulsions to-day. Recovered.
Many hyaline, granular casts; R. B. C.; W. B. C.	None	32.2	14.8	Fifty convulsions in past 24 hours. Comatose. Recovered.
Hyaline, granular casts; W. B. C.	None	39.7	19.9	Typical case. Died 12 hours later.
Occasional R.B.C.; W. B. C.	Mixed	23.1	11.5	Para–7. Eclampsia with fifth pregnancy. Induced labor for threatened eclampsia with sixth pregnancy. Headache; scanty urine. Discharged relieved.
Hyaline, granular casts; W. B. C.	Soft	28.9	14.1	Headache; edema. Discharged relieved.
Hyaline, granular casts; W. B. C.	Liquid	39.7 24.7	21.4 12.6	First blood analysis, 4/15/14. Delivered, 4/18/14. Second analysis, 4/29/14. Recovered.
Hyaline casts; W. B. C.	Soft	20.7	8.0	Vomiting for 9 weeks. Recovered.
Hyaline, granular casts	None	28.7	13.7	Vomiting for 5 weeks. Died same day.

diagnostic value. The differential diagnosis of eclampsia from uremia has been up to the present in a highly unsatisfactory state, but with the aid of blood analysis it should now be possible with a high degree of probability.

In the peculiar condition known as the nephritis of pregnancy, we have found nearly normal values in two cases. Farr and Williams report three cases, of which two were practically normal; the third showed 52 mg. of nitrogen, falling to normal after delivery.

In hyperemesis we have found normal figures in two cases, one of them fatal, and Farr and Williams examined one case with the same result.

V. ACUTE INTESTINAL OBSTRUCTION

In this condition we have found a remarkable increase in the nonprotein nitrogen and urea, as marked as in most cases of uremia. In one of the three cases the obstruction was mechanical, due to a band; in the other two it was of the paralytic variety. In a case of simple constipation examined for the sake of comparison, there was no retention, although the bowels had not moved for three days (Case 57). The complete disappearance of the retention in the two cases ending in recovery is evidence that the azotemia was not due to complicating disease of the kidneys.

TABLE 6.—ACUTE—

Case No.	Sex	Age	Diagnosis	Systolic Blood-Pressure mm. Hg	Urine			
					Phenol-sulphone-phthalein % in 2 hrs.	Amt. c.c. in 24 hours	Sp. Gr.	Alb.
54	F	70	Strangulated hernia	...	25 43	730	1.020	V. S. T.
55	F	26	Acute obstruction	116	..	500+	1.011	S. P. T.
56	M	60	Paralytic ileus, following prostatectomy	140	70	S. S.	1.028	S. T.
57	F	21	Simple constipation	124	77	380+	1.022	S. P. T.

The case of mechanical obstruction (No. 55) showed on the day of operation 92 mg. of nitrogen, and nine days later, although the obstruction had been relieved, 169 mg.; death took place the following day. The postoperative symptoms in this case were delirium, vomiting, apathy and profound asthenia, symptoms strongly suggestive of uremia. No necropsy was permitted.

The two cases of paralytic ileus are very instructive, for they show that it is possible to recover from a very high degree of retention, provided that it is not too prolonged. In Case 56 the symptoms of acute intestinal obstruction developed two days after the operation of prostatectomy by the perineal route. Blood analysis the following day revealed 150 mg. of nitrogen and 92 mg. of urea. In spite of the alarming condition, recovery occurred, and the figures for nitrogen and urea fell to normal.

In the other case of paralytic ileus (No. 54) following an operation for strangulated hernia, the blood was taken after improvement had begun, and showed 76 mg. of nitrogen, falling to normal later; it is probable the retention would have been more marked if the blood had been examined earlier.

The observation of a high degree of retention in acute intestinal obstruction is new, so far as we are aware. It suggests that some of the symptoms, such as profound asthenia and apathy, may be due to the retention of waste products.

In order to explain this striking phenomenon, it is necessary to recall the pathologic physiology of this condition.

—Intestinal Obstruction

Urine		Blood		
Microscopical	Diet	Total N. (mg.)	Urea N. (mg.)	Remarks
Occasional hyaline casts; few R.B.C.; W. B. C.	Liquid Mixed	76.1 29.2	45.5 15.2	Operation, 4/6/14. Paralytic ileus, 4/10/14. Blood taken 3 days later, after improvement had begun. Recovered. Second analysis 2 weeks after first.
Few granular casts; W. B. C.	Liquid	92.2 169.0	72.8 114.0	Acute obstruction due to band, 3 days duration. Relieved by operation, 4/1/14, date of first blood analysis. Mental confusion, apathy, but no convulsions or coma. Second analysis, 4/10/14. Died, 4/11/14.
Few hyaline, granular casts; many W. B. C.	Liquid	150.3 27.6	92.0 15.4	Prostatectomy, 4/9/14, followed by paralytic ileus lasting several days. First blood analysis, 4/12/14; second, 5/1/14. Recovered.
Occasional hyaline cast; W. B. C.	Mixed	30.4	13.9	Duration, 3 days.

There is an enormous increase in the secretion into the intestine, and owing to the stagnation of the intestinal contents, a marked increase in the decomposition of nitrogenous substances, as shown by the large amounts of indican in the urine. There is therefore an opportunity for increased absorption of non-protein nitrogen from the intestine. The profound shock and the possible intoxication by poisonous substances absorbed from the intestine (cf. Whipple[17]) must affect unfavorably the renal function, leading to defective elimination of waste products. And, lastly, the great loss of fluid by vomiting,[18] with the resulting concentration of the blood, is undoubtedly a factor,

17. Whipple, Stone and Bernheim: Jour. Exper. Med., 1913, xvii, 286.

18. Hartwell, John A., Hoguet, J. P., and Beekman, Fenwick: An Experimental Study of Intestinal Obstruction, The Archives Int. Med., 1914, xiii, 701.

though not enough in itself to account for a five-fold increase in the total non-protein nitrogen.

VI. DISEASES OF THE HEART AND AORTA

Three cases of pericarditis with effusion and a case of severe acute endocarditis ending in recovery, all showed normal figures, as did also a compensated case of extensive mitral and aortic valvular disease, and a patient with aortic aneurysm. A typical case of subacute infective endocarditis (Case 63) with anemia and enormous spleen (1035

TABLE 7.—DISEASES OF—

Case No.	Sex	Age	Diagnosis	Systolic Blood-Pressure mm. Hg	Urine			
					Phenol-sulphone-phthalein % in 2 hrs.	Amt. c.c. in 24 hours	Sp. Gr.	Alb.
58	M	8	Pericarditis with effusion, rheumatic	88	80	270	1.022	S. P. T.
59	M	17	Pericarditis with effusion, rheumatic	140	68	740	1.028	S. P. T.
60	M	38	Pericarditis with effusion, tuberculous	110	37	1,280	1.018	V. S. T.
61	F	23	Endocarditis, chronic....	127	63	S. S.	1.019	S. P. T.
62	F	19	Endocarditis, acute.....	146	54	350+	1.019	S. T.
63	M	22	Endocarditis, malignant.	115	45	925	1.011	S. T.
64	M	55	Aneurysm of aorta.....	140	70	600	1.023	V. S. T.

gm.) showed considerable retention. At the necropsy the kidneys showed recent infarcts and multiple pin-point-sized hemorrhages. Microscopically, there were the signs of an acute diffuse nephritis, and many glomeruli showed hemorrhages into the capsular space. One miliary abscess was seen.

The results in cardiac decompensation have been stated already under the heading "chronic passive hyperemia of the kidneys."

VII. THE ACUTE INFECTIONS

A. Pneumonia.—In acute lobar pneumonia we have found a moderate degree of retention in the majority of the cases, nine out of fourteen patients showing more than 35 mg. of nitrogen; the highest figure was 50 mg. The retention reached its maximum toward the

crisis and disappeared early in convalescence. It was not dependent on the absorption of the exudate, for it occurred before resolution took place. The figures were lower in the younger patients, three out of the four under 20 years of age showing normal values. There was no apparent relation between the degree of retention and prognosis.

B. Typhoid Fever. — In typhoid we have met normal or slightly elevated figures in the small series studied (five cases). This is in agreement with the infrequency of severe lesions of the kidney in typhoid.

Urine		Blood		
Microscopical	Diet	Total N. (mg.)	Urea N. (mg.)	Remarks
Few hyaline casts; W. B. C.	Soft	22.7	11.0	Chronic mitral disease. Recovered.
W. B. C.	Soft	23.2	11.5	Chronic mitral and aortic disease. Developed pleural effusion and pneumonia later. Recovered.
Occasional hyaline cast; R. B. C.; many W. B. C.	Soft	29.2	15.7	Lymphocytosis in pericardial fluid. Tapped several times.
Hyaline casts; few R. B. C.; many W. B. C.	Mixed	30.2	14.8	Mitral and aortic stenosis and insufficiency, compensated. Rheumatic.
Occasional hyaline cast; R. B. C.; W. B. C.	Mixed	30.2	16.1	Acute aortic endocarditis, with fever lasting 7 weeks. Recovered.
Few hyaline, granular, pus casts; R. B. C.; W. B. C.	Soft	68.0	42.5	Typical subacute infective endocarditis. At necropsy, kidneys showed recent infarcts and acute diffuse nephritis.
Occasional hyaline cast; W. B. C.	Soft	30.9	16.1	No failure of compensation.

C. Acute Articular Rheumatism.—This has yielded very low figures, probably because of the small amount of nourishment taken by these patients and the lack of renal involvement.

D. Scarlet Fever.—In scarlatina, contrary to our expectations, there was not the slightest retention in any of the four cases studied. None of these, to be sure, was very severe.

VIII. SYPHILIS

We have examined eleven cases of this disease, two in the primary, four in the secondary and five in the tertiary stage. In every case in which the urine was examined, albumin and casts were found.

Of the primary cases, one was normal, the other moderately elevated (36 mg.).

TABLE 8.—Acute—

Case No.	Sex	Age	Diagnosis	Day of Disease	Systolic Blood Pressure mm. Hg	Urine			
						Phenolsulphonephthalein % in 2 hrs.	Amt. c.c. in 24 hours	Sp. Gr.	Alb.
65	M	17	Pneumonia, acute lobar	5	140	..	S. S.	1.023	0.1%
66	M	9	Pneumonia, acute lobar	4	85	68	365	1.024	S. P. T.
				7	...	..	....	.....	.....
67	F	16	Pneumonia, acute lobar	5	125	35	750	1.026	0.05%
				7	...	..	....	.....	.....
				9	...	..	....	.....	.....
68	M	33	Pneumonia, acute lobar	..	...	50	450	1.017	S. P. T.
69	M	27	Pneumonia, acute lobar	6	...	80	1,520	1.016	S. P. T.
70	M	36	Pneumonia, acute lobar	8	108	30	670	1.024	S. T.
				16	...	..	....	.....	.....
71	M	28	Pneumonia, acute lobar	4	135	..	770	1.024	S. P. T.
72	M	39	Pneumonia, acute lobar	4	108	..	465+	1.026	S. P. T.
73	M	39	Pneumonia, acute lobar	6	85	..	450	1.017	S. P. T.
74	M	25	Pneumonia, acute lobar	8	120	60	850	1.026	S. P. T.
				15	...	..	....	.....	.....
75	M	51	Pneumonia, acute lobar	8?	140	..	1,730	1.016	S. T.
76	M	48	Pneumonia, acute lobar	13?	160	..	680	1.020	S. P. T.
				16?	...	..	....	.....	.....
77	M	15	Pneumonia, acute lobar	..	88	80	385	1.022	V. S. T.
78	F	40	Pneumonia, acute lobar	10	102	41	S. S.	1.020	V. S. T.
				12	...	..	....	.....	.....
79	F	18	Typhoid fever	16	100	61	730	1.016	S. P. T.
80	M	15	Typhoid fever	19	105	..	730	1.021	S. P. T.
						62	....	.....	.....
81	M	36	Typhoid fever	17	105	67	1,040	1.017	V. S. T.
82	F	10	Typhoid fever	12	115	71	270	1.020	V. S. T.
83	M	30	Typhoid fever	20	90	..	S. S.	1.025	0.05%
84	F	23	Acute articular rheumatism	3	110	..	700	1.020	S. P. T.
85	M	28	Acute articular rheumatism	16	112	70	640	1.026	S. P. T.
86	F	23	Erythema nodosum ...	21	100	..	575	1.020	S. P. T.
87	F	19	Puerperal sepsis	..	102	50	350	1.013	0.15%
88	M	19	Scarlatina	5	...	..	S. S.	1.025	S. P. T.
89	F	10	Scarlatina	4	...	..	870	1.018	S. P. T.
90	M	23	Scarlatina	4	...	..	800	1.025	S. P. T.
91	M	14	Scarlatina	4	...	..	825	1.018	S. P. T.
92	M	19	Measles	6	...	45	1,670	1.020	S. P. T.
93	M	18	Meningococcus meningitis	..	138	50	835	1.021	S. P. T.
94	M	23	Appendicitis with retrocecal abscess	2	115	..	S. S.	1.025	0.0

*The typhoid diet employed is a generous one of about 3,000 calories, containing

Urine	Diet	Blood		Remarks
Microscopical		Total N. (mg.)	Urea N. (mg.)	
Many brown granular casts; R. B. C.; W. B. C.	Liquid	23.2	11.2	Died on seventh day.
W. B. C.	Soft	23.1	11.8	Crisis ended next day. Moderately severe case. Recovered.
		24.0	12.6	
Few hyaline, granular casts	Liquid	27.0	14.0	First normal temperature on ninth day. Severe case. Recovered.
		23.0	13.4	
		28.5	13.4	
Few granular casts; W. B. C.	Soft	28.1	14.3	Died 2 days later.
Occasional hyaline cast; W. B. C.	Soft	30.0	16.6	Recovered.
Granular casts; W. B. C.	Soft	31.7	....	Acute followed by chronic pneumonia and failure of resolution. Recovered.
		40.5	27.6	
W. B. C.	Soft	33.5	18.4	Crisis next day. Recovered.
Many hyaline casts; W. B. C.	Soft	36.2	19.1	Recovered.
Many granular casts; R. B. C.; W. B. C.	Soft	39.2	30.3	Died 4 days later.
Hyaline, granular casts R. B. C.; W. B. C.	Soft	40.2	26.9	
		32.4	19.0	Recovered.
Hyaline, granular casts; W. B. C.	Soft	41.0	27.8	Died 13 days later.
Granular casts; W. B. C.	Soft	41.5	24.1	Died 6 days after first blood analysis.
		44.0	31.7	
Hyaline, granular casts; W. B. C.	Soft	43.7	30.9	3/6/14.
		50.0	36.6	3/9/14. After crisis.
		31.2	19.6	3/13/14. Crisis 2 days after first analysis. Recovered.
Hyaline, granular casts; W. B. C.	Liquid	46.2	30.0	Complicated with jaundice. Crisis on eleventh day. Recovered.
		44.0	25.3	
No casts; few R. B. C.; W. B. C.	Soft	21.1	10.1	Temp. 103.8 F. Moderately severe.
Rare hyaline cast; R. B. C.; W. B. C.	Typhoid*	27.0	13.7	4/ 4/14. Temp. 104 F.
		25.6	12.9	4/27/14. Temp. 100 F. Moderately severe.
Occasional hyaline cast; few R.B.C.; many W. B. C.	Soft; forced liq.	27.2	13.3	Temp. 102 F. Moderately severe.
Few hyaline casts; few R.B.C.; many W. B. C.	Typhoid*	29.2	15.8	Temp. 103 F. Moderately severe.
Many hyaline, granular casts	Liquid	33.5	19.2	Temp. 103 F. Died on thirty-ninth day of disease.
W. B. C.	Soft	19.6	9.4	Moderately severe.
Hyaline casts; rare R. B. C.; W. B. C.	Soft	27.4	15.4	Moderately severe.
Many W. B. C.	Liquid	22.7	11.5	Prolonged fever.
Pus	Soft, with meat once daily	22.5	11.1	Recovered.
Occasional hyaline cast; R. B. C.	Moderate protein.†	21.2	11.7	Mild case.
Negative	Soft	23.2	10.9	Moderately severe.
W. B. C.	Mixed	27.7	13.8	Moderately severe.
Negative	Soft	29.1	14.1	Mild case.
Negative	Mixed	27.4	18.4	
R. B. C.; W. B. C.	Soft	27.3	15.6	Severe case. Treated with serum. Temp. 104 F. on day of analysis. Recovered.
No casts; few R. B. C.; many W. B. C.	Soft	29.7	18.0	

100 gm. protein. † No meat or fish.

The majority of those in the secondary stage was also normal, only one showing an increase (41 mg.).

In the tertiary stage there was more evidence of retention, one case showing 51 mg., another 55 mg. of nitrogen, while the remaining three were normal.

For the whole series we find 36 per cent. with a total non-protein nitrogen of 35 mg. or more. Folin and Denis,[19] in a much larger series of 63 cases, reported 46 per cent. showing 35 mg. or more; their highest figure was 45 mg. Their patients were in all stages of the disease; no clinical details are given.

TABLE 9.—

Case No.	Sex	Age	Diagnosis	Systolic Blood-Pressure mm. Hg	Urine			
					Phenol-sulphone-phthalein % in 2 hrs.	Amt. c.c. in 24 hours	Sp. Gr.	Alb.
95	M	26	Primary	102	64	1,150	1.013	V. S. T.
96	M	34	Primary	...	70	1,300	1.017	V. S. T.
97	M	28	Secondary	...	..	.....	.....	.....
98	F	38	Secondary	...	..	.....	.....	.....
99	F	24	Secondary	...	..	.....	.....	.....
100	M	47	Secondary, early	...	..	.....	.....	.....
101	M	46	Tertiary	130	..	460	1.026	V. S. T.
102	M	42	Tertiary	160	60	910	1.030	0.2%
103	F	45	Tertiary	110	..	(?) 220	1.023	S. P. T.
104	M	28	Tertiary	...	..	S. S.	1.015	S. P. T.
105	M	53	Tertiary	...	..	S. S.	1.024	S. P. T.

From these data it seems fair to conclude that the kidneys are affected in a very considerable proportion of cases in all stages of the disease. This involvement is probably temporary as a rule, but may often go on to permanent changes in the kidney, usually of the interstitial type. Further investigation along these lines is desirable. The effect of antisyphilitic treatment, especially salvarsan, should be studied.

19. Folin, O., and Denis, W.: Jour. Biol. Chem., 1913, xiv, 29.

IX. TUBERCULOSIS

Out of three cases of pulmonary tuberculosis, all advanced and active, two showed a considerable retention. The study of a large series should yield interesting results.

X. DISEASES OF THE NERVOUS SYSTEM

Cerebral hemorrhage (including one case of skull fracture) has not been accompanied by any notable azotemia. It is thus possible to distinguish between apopleptic and uremic coma by means of blood analysis.

—Syphilis

Urine		Blood		
Microscopical	Diet	Total N. (mg.)	Urea N. (mg.)	Remarks
.....	Mixed	29.5	14.9	Chancre 3 weeks ago. Wassermann ++. Untreated.
Hyaline, fine granular casts; W.B.C.	Mixed	36.0	19.6	One dose of neosalvarsan, 16 days ago.
.....	Mixed	23.7	14.0	Chancre 1 year ago. Now mucous patches in mouth. Untreated.
.....	Mixed	24.1	10.4	Sore throat for 2 months. Mucous patches on tongue and tonsils; spirochetes in same. Untreated.
.....	Mixed,low	26.8	15.1	Flat papular syphilid, generalized. Salvarsan 6 days ago.
.....	Mixed	41.0	21.3	Chancre on chin. Roseola. Untreated.
Few granular casts	Soft	25.7	12.8	Epileptiform seizures, followed by paralysis of arm. Blood and spinal Wassermann negative.
Granular casts; W. B. C.	Purin free	28.0	....	1/24/14. Aneurysm of aorta; nephrolithia-
		26.2	13.7	2/ 4/14. sis. Wassermann +.
Rare hyaline cast; W. B. C.	Mixed	28.5	14.0	Obstinate headaches; relieved later by salvarsan.
Hyaline, granular casts; R. B. C. W. B. C.	Mixed	51.2	28.1	Periostitis. Infection 14 years ago.
Hyaline, granular casts; W. B. C.	Mixed, little meat	55.0	35.9	Extensive ulceration of soft palate. Duration 1 year. Liver and spleen both enlarged.

Neurasthenia and hysteria have shown normal values, as was to be expected. The three cases of hysteria were all studied immediately after an attack of convulsions.

XI. MISCELLANEOUS DISEASES

A. The Anemias.—Three of the four cases of anemia have shown a considerable degree of azotemia. In two of these hemolysis was very evident, and the retention may perhaps be explained by the clog-

ging of the tubules of the kidney with hemoglobin and disintegrated red cells, such as has been demonstrated in fatal cases of "black-water fever." This would apply especially to Case 121, in which hemoglobinuria and reduction of the red count to one million occurred in a case of atypical lobar pneumonia due to the pneumococcus. In the other case of hemolytic anemia (No. 122) a woman with one million red cells, slight jaundice and an enlarged spleen, the nitrogen reached the very high figure of 109 mg., and the phenolsulphonephthalein output was nil. There was stupor, but no other sign of uremia. With improvement in the blood condition the nitrogen content returned to normal, and the phenolsulphonephthalein excretion rose to 31 per cent.

A case of polycythemia showed 44 mg. of nitrogen.

TABLE 10.—

Case No.	Sex	Age	Diagnosis	Systolic Blood-Pressure mm. Hg	Urine			
					Phenol-sulphone-phthalein % in 2 hrs.	Amt. c.c. in 24 hours	Sp. Gr.	Alb.
106	F	32	Pulmonary	140	50	600+	1.020	S. P. T.
107	M	49	Pulmonary	133	63	1,220	1.020	S. P. T.
108	M	21	Pulmonary	100	..	560	1.027	S. P. T.

B. Diabetes.—Uncomplicated cases have shown no retention. Two typical cases of diabetic coma have shown a considerable azotemia, up to 67 mg. of nitrogen, which is not surprising in such a grave disturbance of metabolism. A third case, however, with deep breathing and on the verge of coma, showed normal figures (Case 126).

C. Gout.—Both cases had a moderate retention, which can be referred to involvement of the kidneys in the gouty process.

D. Myxedema.—Each of the two cases of myxedema was complicated with chronic nephritis. Curiously enough, the one with very high blood-pressure showed normal values, while the other, with normal blood-pressure, had a marked azotemia (65 mg. nitrogen). This is a good illustration of the absolute lack of correspondence between the blood-pressure and the non-protein nitrogen. Folin and Seymour[20] have called attention to this point.

20. Folin, O., Denis, W., and Seymour, M.: The Non-Protein Nitrogenous Constituents of the Blood in Chronic Vascular Nephritis (Arteriosclerosis) as Influenced by the Level of Protein Metabolism, THE ARCHIVES INT. MED., 1914, xiii, 224.

The influence of thyroid secretion on metabolism is shown by the considerable rise in non-protein nitrogen and urea in both these cases when thyroid extract was administered. The increase averaged 6.1 mg. for the nitrogen and 4.5 mg. for the urea.

E. Exophthalmic Goiter.—Normal values were found in one case, and also in another complicated with diabetes (Case 128).

F. Malignant Disease.—One of the three cases of carcinoma showed a considerable retention; the other two were normal.

G. Acute Yellow Atrophy of the Liver.—We have examined one case of this disease (No. 137), typical in all respects except in the termination in recovery. During the height of the symptoms there was a considerable retention of waste nitrogen. The chief point of

—Tuberculosis

Urine		Blood		
Microscopical	Diet	Total N. (mg.)	Urea N. (mg.)	Remarks
Few hyaline casts; R. B. C.; W. B. C.	Mixed	22.9	12.7	Temp. 101 F. Third stage.
Hyaline, granular casts; R. B. C.; W. B. C.	Soft	40.0	26.2	Febrile. Second stage. Profuse hemorrhage 11 days previous to analysis.
Occasional hyaline cast; W. B. C.	Soft	43.5	19.9	Febrile. Second stage.

interest, however, lies in the urea, which at the first two examinations was decidedly below the normal, constituting 32 per cent. and 41 per cent. of the total nitrogen, respectively, while usually with retention there is an increase in the urea fraction, often up to 70 or 80 per cent. of the whole. This phenomenon is in accord with the diminished excretion of urea, and the supposed diminished urea formation in the liver in this disease. The last analysis, made as the patient was beginning to improve, though still deeply jaundiced, showed nearly normal figures.

The Effect of Diet in Azotemia.—Folin, Denis and Seymour[20] have investigated the effect of a protein-poor diet in chronic interstitial nephritis, and found in every case a marked reduction in both nitrogen and urea, usually though not always to normal or below. Their highest figure for nitrogen was 58 mg. We also have experimented in this way in a few cases, using the protein-poor diet recommended by Goodall.[21] In two cases (Nos. 12 and 36) a considerable retention

21. Goodall, H. W.: Boston Med. and Surg. Jour., 1913, clxviii, 760.

of about 50 mg. nitrogen was caused to disappear by diet. In uremic patients with excessively high figures (cf. Case 16) we have not been able to reduce the azotemia to any important degree.

Prognosis and Blood Analysis.—In chronic nephritis and obstruction of the urinary tract resulting from urethral stricture and cancer of the bladder, we have found that a nitrogen value of over 90 mg. has been followed by death within a period of thirty-five days in all but one of ten cases. The exception was Case 13, previously referred to. Four patients with over 200 mg. of nitrogen all died in nineteen days or less. When, however, the condition leading to excessive reten-

TABLE 11.—DISEASES—

Case No.	Sex	Age	Diagnosis	Systolic Blood-Pressure mm. Hg	Urine			
					Phenol-sulphone-phthalein % in 2 hrs.	Amt. c.c. in 24 hours	Sp. Gr.	Alb.
109	M	63	Fracture of skull with cerebral hemorrhage	180	30	S. S.	1.025	V. S. T.
110	M	60	Cerebral hemorrhage	205	59	S. S.	1.030	S. P. T.
111	F	53	Cerebral hemorrhage	220	50	S. S.	1.027	0.2%
112	M	52	Cerebral hemorrhage	190	..	S. S.	1.020	H. T.
113	F	43	Neurasthenia	140	..	S. S.	.....	S. P. T.
114	M	54	Neurasthenia	140	..	S. S.	1.022	S. P. T.
115	F	48	Neurasthenia	140	..	S. S.	1.014	S. P. T.
116	F	28	Hysteria	...	..	S. S.	1.012	T.
117	F	38	Hysteria	...	..	S. S.	1.004	S. P. T.
118	F	30	Hysteria	135	50	550	1.023	S. P. T.

tion is temporary, as, for instance, in acute intestinal obstruction (Case 56), or hemolytic anemia (Case 122), a total nitrogen value of even 150 mg. is not inconsistent with recovery.

SUMMARY AND CONCLUSIONS

Analyses of the blood for total non-protein nitrogen and urea nitrogen have been made by the methods of Folin in five healthy adults and 142 hospital cases.

1. In the fasting healthy adult the total non-protein nitrogen varied betweeen 22.9 and 25 mg. per 100 c.c. of blood, and the urea nitrogen between 12 and 14 mg.

2. The effect of a full meal with meat in the case of the healthy adult was a rise of total non-protein nitrogen averaging 4.7 mg., and of urea, averaging 2.5 mg.

3. In both chronic interstitial and chronic diffuse nephritis the cases without symptoms of uremia showed normal or moderately elevated values; the uremic cases, with one possible exception, showed a great increase in both nitrogen and urea.

4. The excretion of phenolsulphonephthalein was roughly proportionate to the degree of retention; the cases with 100 mg. or over of total nitrogen all showed 5 per cent. or less phenolsulphonephthalein excretion. Many cases, however, with a considerable impairment of

Urine		Blood		
Microscopical	Diet	Total N. (mg.)	Urea N. (mg.)	Remarks
R. B. C.; W. B. C.	None	20.2	10.0	Convulsions. Fatal case.
Few hyaline, granular casts	Soft	27.0	13.7	Recovered.
Hyaline, granular casts; W. B. C.	Liquid	28.7	14.1	Died 45 days later.
Hyaline, granular casts	.....	36.1	18.6	Died few hours later.
No casts	Mixed	22.7	11.5	
No casts	Mixed	25.7		
No casts	Mixed	27.8	13.5	
Numerous hyaline, granular casts; W. B. C.	Mixed	22.0	12.0	Hysterical convulsions.
No casts	Mixed	26.5	14.4	Hysterical convulsions.
Hyaline casts; W. B. C.	Soft	27.2	12.0	Hysterical convulsions.

phenolsulphonephthalein excretion showed no signs of retention, and a moderate amount of retention of waste nitrogen often occurred with no impairment of the elimination of phthalein.

5. The proportion of urea nitrogen to the total non-protein nitrogen in disease varied from 32 per cent. to 85 per cent. Where the nitrogen was normal the urea usually was about one-half the total nitrogen; where it was elevated, the urea usually but by no means always constituted about 70 per cent. of the whole. No reasons could be found for these variations. The determination of the total non-protein nitrogen alone is therefore more valuable than that of the urea alone.

TABLE 12.—MISCELLANEOUS—

Case No.	Sex	Age	Diagnosis	Systolic Blood-Pressure mm. Hg	Urine			
					Phenol-sulphone-phthalein % in 2 hrs.	Amt. c.c. in 24 hours	Sp. Gr.	Alb.
119	F	39	Anemia, secondary	115	66	640	1.020	V. S. T.
120	M	28	Anemia, secondary	125	69	760	1.022	V. S. T.
121	M	45	Anemia, hemolytic	135	..	2,640	1.012	0.2%
122	F	25	Anemia, hemolytic	120	0	700	1.020	S. T.
					31	820	1.020	S. P. T.
123	M	18	Anemia, Banti's disease..	...	33	S. S.	1.015	S. P. T.
124	M	43	Polycythemia	130	..	1,530	1.012	S. P. T.
125	F	59	Diabetes	155	80	.....	.....	.....
126	M	17	Diabetes	95	63	5,370	1.017	V. S. T.
127	F	55	Diabetes	165	80	1,500	1.023	S. P. T.
128	F	37	Diabetes, with exophthalmic goiter	...	75	1,840	1.019	S. P. T.
129	M	64	Diabetes, with anemia...	145	47	1,360	1.020	S. P. T.
130	M	40†	Diabetes, with coma.....	...	10	1,220+	1.024	S. P. T.
131	M	44	Diabetes, with coma.....	...	..	S. S.	.....	S. T.
132	M	50	Gout	150	..	500	1.030	S. P. T.
133	M	73	Gout	117	60	1,080	1.021	S. P. T.
134	F	66	Myxedema	215	58	1,640	1.010	S. P. T.
135	F	61	Myxedema	120	30	1,280	1.014	S. P. T.
136	F	36	Exophthalmic goiter	128	65	700	1.023	S. P. T.
137	M	23	Acute yellow atrophy....	120	..	S. S.	1.026	V. S. T.
138	F	43	Cirrhosis of liver.....	...	..	S. S.	1.012	V. S. T.
139	M	49	Carcinoma of liver, primary	...	..	400+	1.022	V. S. T.
140	M	65	Carcinoma of pancreas...	130	..	S. S.	1.018	S. T.
141	M	66	Carcinoma of pancreas...	115	..	500	1.021	S. T.

† About.

Urine		Blood		
Microscopical	Diet	Total N. (mg.)	Urea N. (mg.)	Remarks
Occasional hyaline cast; few R.B.C.; W. B. C.	Mixed	30.4	14.8	Hb., 10-15 %; R. B. C., 2,160,000; nucleated reds. Uterine hemorrhage following abortion.
W. B. C.	Soft	50.0	28.1	Hb., 15%; R. B. C., 1,460,000; nucleated reds. Profuse hemorrhage from stomach and intestine shortly before analysis, duodenal ulcer.
Numerous hemaglobin casts; rare R. B. C.	Liquid	83.7	59.7	Atypical acute lobar pneumonia, complicated with hemoglobinuria setting in on twelfth day, and lasting 3 days. Blood analysis on fifteenth day. Red count, 1,072,000. Died on twenty-first day.
Few hyaline casts; few R. B. C.; W. B. C.	Soft	108.8	79.0	6/26/14 Hb., 20%; R.B.C., 780,000; W.B.C. 32,000.
Few hyaline casts; few R. B. C.; W. B. C.	Mixed	26.1	....	7/17/14 Hb., 60%; R.B.C., 2,820,000; W.B.C. 6,200. At first analysis, nucleated reds; increased fragility of red cells; enlarged spleen and slight jaundice.
Few hyaline casts; W. B. C.	Refused food	39.7	21.3	Hb., 35%; R.B.C., 2,216,000. Died 2 days later.
No casts; occasional R. B. C.	Mixed	43.7	24.3	R.B.C., 7,900,000. Enlarged spleen with cyanosis.
.....	Janeway	21.1	13.6	Sugar, 2.3%. Moderately severe case.
Hyaline, granular casts; R. B. C.	Janeway	25.0	9.0	Acidosis with deep breathing. Died in coma 4 days later.
Occasional hyaline cast; W. B. C.	Janeway	27.5	16.7	Sugar, 5.0% on entrance; 0.5% on day of blood analysis. Moderately severe case.
Hyaline, granular casts; R. B. C.; W. B. C.	Janeway	27.7	13.7	Sugar, 4.5% on entrance; 0.7% on day of analysis. Developed acidosis 2 days later and died suddenly the same day, not in coma.
Rare hyaline cast; W. B. C.	Janeway	29.4	13.8	Probably pancreatic diabetes. Blood-count: Hb., 35%; R.B.C., 2,400,000; no nucleated reds.
Hyaline, granular casts; R. B. C.; W. B. C.	None	45.8	23.3	Typical acid coma. Died 36 hours after blood was taken.
.....	None	66.9	44.0	Large amount of sugar. Typical acid coma. Died few hours after blood was taken.
Hyaline casts; W. B. C.	Soft	35.1	21.2	Two attacks a number of years previous. Tophus in ear.
Hyaline, granular casts; R. B. C.	Soft	38.9	27.0	Typical attacks for many years.
Hyaline, granular casts; W. B. C.	Mixed	23.5	11.5	Without thyroid extract.
		31.2	13.2	With thyroid, 9 days.
Hyaline, granular casts; W. B. C.	Mixed	60.2	39.8	Without thyroid extract.
		64.7	47.0	With thyroid. No renal symptoms.
Hyaline casts; many R. and W. B. C.	Soft	27.7	13.5	Marked symptoms of hyperthyroidism.
Occasional hyaline, granular casts; R. B. C.; W. B. C.	Liquid	30.4	9.7	5/22/14 Jaundice with delirium, mania
		42.0	17.1	5/24/14 and stupor. Liver much de-
		44.7	19.5	5/25/14 creased in size. Leucin and
		44.2	23.4	5/26/14 tyrosin in urine. Complete recov-
		33.1	15.0	5/27/14 ery with return of liver to normal size.
Rare hyaline cast; occasional W.B.C.	Mixed	21.2	8.8	Large liver and spleen without ascites. Alcoholism. Positive Wassermann.
Rare granular cast	Mixed	27.0	12.9	Died 12 days later. Diagnosis proved by necropsy.
Hyaline, granular casts; W. B. C.	Fat-free	33.5	13.0	Jaundice with dilated gall-bladder, and typical pancreatic stools.
Hyaline, granular casts; W. B. C.	Soft	41.4	22.6	Jaundice. Carcinoma of head of pancreas at necropsy.

6. The estimation of the total non-protein nitrogen is of the greatest value in the diagnosis of uremia. Amounts of 100 mg. or over were encountered in only two conditions besides uremia, namely, acute intestinal obstruction and profound anemia from hemolysis. Only one case of uremia without marked increase in nitrogen was encountered out of a total of eight cases.

7. The determination of the total non-protein nitrogen is a great aid in the prognosis of chronic nephritis. Patients showing over 100 mg., with one exception, did not live more than thirty-five days.

8. The results of blood analysis furnish the best guide as to the diet to be given in nephritis; cases with a considerable retention require a restriction of the protein, and by this means a return to normal figures may be brought about, if the azotemia is not too pronounced. In cases of outspoken uremia, however, no marked reduction of the azotemia has resulted from a protein-poor diet. Nephritis with a normal amount of non-protein nitrogen does not call for any *marked* decrease of protein in the diet.

9. In chronic passive congestion of the kidneys there is little or no retention of nitrogenous waste products.

10. In pyelitis the presence of azotemia probably indicates involvement of the parenchyma of the kidney.

11. A marked elevation of the non-protein nitrogen or urea renders the patient a poor operative risk, and the azotemia should be overcome by diet, if possible, before an operation is attempted, in all cases in which delay is permissible. In hypertrophy of the prostate, for example, a low-protein diet may be combined with drainage of the bladder as a preliminary to operation.

12. Chronic lead-poisoning was accompanied by evidence of retention in all the cases examined.

13. The eclampsia of pregnancy seldom shows a marked increase in non-protein nitrogen and urea. It is therefore distinct from uremia. Analysis of the blood will usually serve to distinguish between uremia and eclampsia.

14. In acute intestinal obstruction a tremendous increase in the nitrogenous waste products was found in all of the three cases examined. A return to normal took place in the two which recovered.

15. Compensated valvular disease of the heart, aortic aneurysm. acute pericarditis with effusion, and acute endocarditis in the absence of disease of the kidneys, all showed normal values.

16. In acute lobar pneumonia a considerable increase was seen in the majority of cases, reaching its maximum toward the crisis, but bearing no relation to the time at which resolution took place. Typhoid

fever, acute rheumatism and uncomplicated scarlatina showed normal figures.

17. Syphilis showed a considerable degree of retention in 36 per cent. of the cases examined, evident in all stages of the disease.

18. In cerebral hemorrhage, hysteria and neurasthenia no increase was found.

19. Severe anemia due to hemolysis showed a marked retention, reaching in one case 100 mg. of nitrogen.

20. In uncomplicated diabetes the values were normal; both cases examined in coma showed retention.

21. The administration of thyroid extract in two cases of myxedema caused an increase in both nitrogen and urea. Both cases were complicated with chronic nephritis.

22. In exophthalmic goiter the blood nitrogen and urea were normal in amount.

23. No changes were met with in malignant disease which could not be ascribed to a complication with renal disease.

24. In a case of acute yellow atrophy the proportion of urea nitrogen to the total non-protein nitrogen was decreased, although there was a considerable degree of azotemia.

In conclusion we take pleasure in acknowledging our indebtedness to Professor Folin, Dr. Henry Lyman and Dr. Richard P. Bell for helpful advice in the laboratory work, and to the staff of the New Haven Hospital for placing the clinical material at our disposal.

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AN EPIDEMIC OF STREPTOCOCCUS SORE THROAT IN JACKSONVILLE, ILL., WHICH WAS TRACED TO THE MILK OF COWS AFFECTED WITH STREPTOCOCCUS MASTITIS *

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During the past winter there occurred in Jacksonville, Ill., a city of 15,000 people, an unusual number of cases of sore throat. In order to check the spread of the disease, twenty-five physicians volunteered their services for the systematic examination of all schoolchildren. The epidemic began soon after Thanksgiving Day, 1913, and reached its height in the month of December. In January many persons were on the sick list, but comparatively few new cases were reported. Early in February the number of cases of tonsillitis was not much above the average of other winters. At the request of local physicians the State Food Commission made an investigation of the milk-supply, but it reported nothing to indicate a causal relationship between the milk and the epidemic.

Later in January, through the kindness and cooperation of the local physicians and the city health commissioner, we were enabled to study the epidemic from a clinical as well as an etiological point of view.

The plan of procedure was as follows:

1. A clinical investigation of cases of sore throat and the relation to the milk-supply.
2. Inspection of the cows, dairies and distributing plants.
3. Bacteriological examinations of throat swabs from infected individuals and of milk from cows showing signs of garget.

1. CLINICAL INVESTIGATION

SYMPTOMATOLOGY

The outbreak was quite abrupt in the last days of November, rapidly reaching a climax December 4 (Chart 1). After a brief recession there occurred a second prolonged rise in the number of cases that persisted for about two weeks. Thereafter the new cases appeared irregularly.

Altogether, 348 cases of sore throat were reported between Nov. 29, 1913, and Feb. 1, 1914.

* Submitted for publication July 10, 1914.

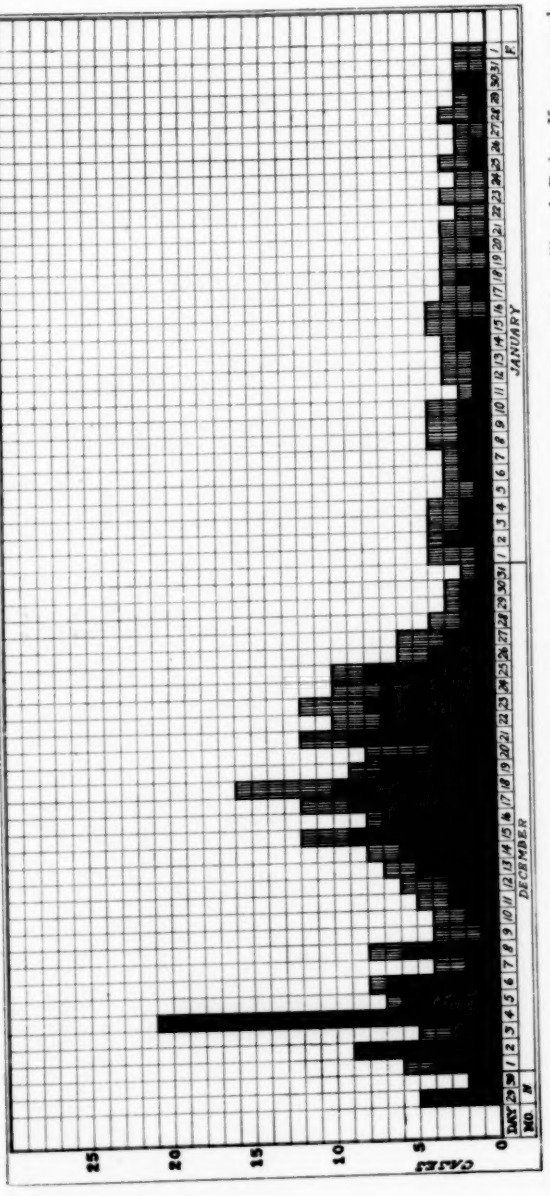

Chart 1.—Outbreak of cases of sore throat by days. Black squares are cases in which milk of Dairy X was used. Lined squares are cases in which milk of other dairies was used.

A definite tonsillitis was described in 217 cases, with appearance of exudate in 104 instances. In addition to a number of cases of true diphtheria with Klebs-Loeffler bacilli, there were 17 cases with an exudate so closely resembling diphtheria that antitoxin was administered. In the latter group Klebs-Loeffler bacilli were not found.

Enlargement of the cervical glands was one of the most constant symptoms, occurring in 192 persons. Among other complications were 5 cases of peritonsillar abscess, 10 of otitis media, 1 of nephritis, and 14 of various skin eruptions, not including 4 cases of erysipelas. Rheumatism of an acute or subacute type was described in 16 cases, and endocarditis in 6.

The duration of the disease gives some idea of its unusual severity and persistence. The average period of illness in nearly 200 cases recorded was between nine and ten days.

Three patients died, two from septicemia and one from peritonitis developing soon after tonsillitis.

Relation of Sore Throat to Milk

(*a*) In a house-to-house canvass carried out by Drs. Thompson and Black, a special inquiry was made concerning the use of milk, cream or ice cream by the sore throat victims and concerning the dairy supplying each household.

In a total of 913 households investigated, 226 histories of sore throat were obtained. In 135 of these cases milk, cream or ice cream was used from a dairy that will be called "Dairy X." Since Dairy X supplied only 160 out of the 913 households, or 17.5 per cent., it is evident that its contribution of 135 out of a total of 226 cases, or 60 per cent., is excessive.

TABLE 1.—Comparative Morbidity and the Milk Supply in the Several Institutions

Institution	Number Residents	Number Cases Sore Throat	Percentage Affected	Dairy
Blind	198	5	2.5	Own
Deaf and dumb	478	34	7.1	Own
Insane	1,725	1	.05	Own
College	200	80	40.0	X
Hospital	50	2	4.0	A

(*b*) There are three large state institutions in Jacksonville, all using milk from their own dairy farms. The physicians in charge furnished us with accurate data on the incidence of sore throat with the approximate time of onset. Similar information was secured

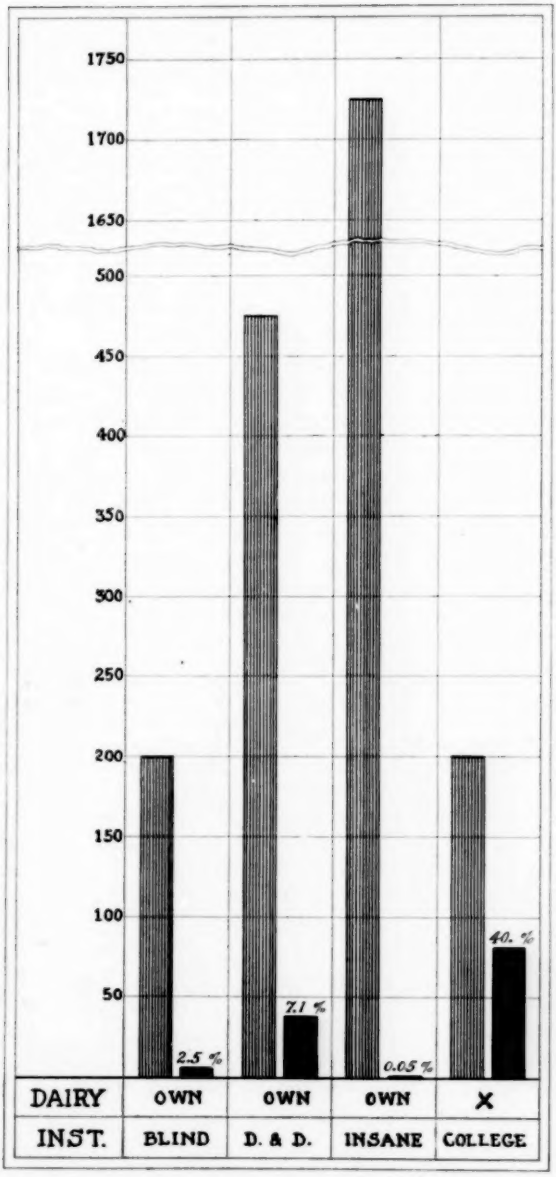

Chart 2.—Number of residents in several large institutions, the comparative prevalence of sore throat and the milk supply of each. Lined columns = number of residents. Black columns = number of cases of sore throat. Note the high morbidity in the college supplied by Dairy X.

regarding the occupants of a college dormitory and the permanent residents of a hospital. Both of the latter institutions depended on outside sources for milk.

Table 1 and Chart 2 bring out sharply the high morbidity in the college. Most of the students were attacked between November 29 and December 21. On one day eighteen were taken ill. In a total of 200 students, eighty, or 40 per cent., were victims.

The college was supplied with milk and cream by the aforementioned Dairy X.

The morbidity in the four other institutions varied from less than 1 per cent. to 7.1 per cent., averaging 1.7 per cent.

An interesting situation at the Insane Hospital should be noted. Twenty-five attendants and other employees, of a total of 220, consulted the house physician for sore throat. These persons often had occasion to eat and drink outside the hospital premises. In spite of close daily contact of these attendants with their charges, only one of the 1,725 patients contracted sore throat during December and January. The element of contagion from one person to another seems to have been negligible.

Again, it may be emphasized that the morbidity of sore throat in the college supplied by Dairy X was over twenty times that of the four other institutions supplied by other dairies.

When a summary is made of 7,416 individuals investigated both in households and institutions, we find that approximately 1,000, or 13.5 per cent., were customers of Dairy X. Of the total of 348 cases of sore throat 215, or 62 per cent., were users of Dairy X milk (Table 2).

TABLE 2.—SUMMARY OF INVESTIGATION

Total number of individuals investigated	7,416
Number of individuals using milk of Dairy X	1,000
Number of individuals using milk of other dairies	6,416
Total number of cases of sore throat	348
Cases of sore throat using milk of Dairy X	215
Cases of sore throat using milk of other dairies	133
Percentage of individuals supplied by Dairy X	13.5
Percentage of cases of sore throat using milk of Dairy X	62.0

Furthermore, among the 6,416 customers of other dairies, 133, or 2.1 per cent., developed sore throat, while among the 1,000 customers of Dairy X 215, or 21.5 per cent., contracted the disease (Chart 3).

2. INSPECTION OF COWS AND DAIRIES

For the inspection of the cows and dairies we were fortunate in securing the services of Mr. Kruger, an experienced dairy inspector in the Chicago health department.

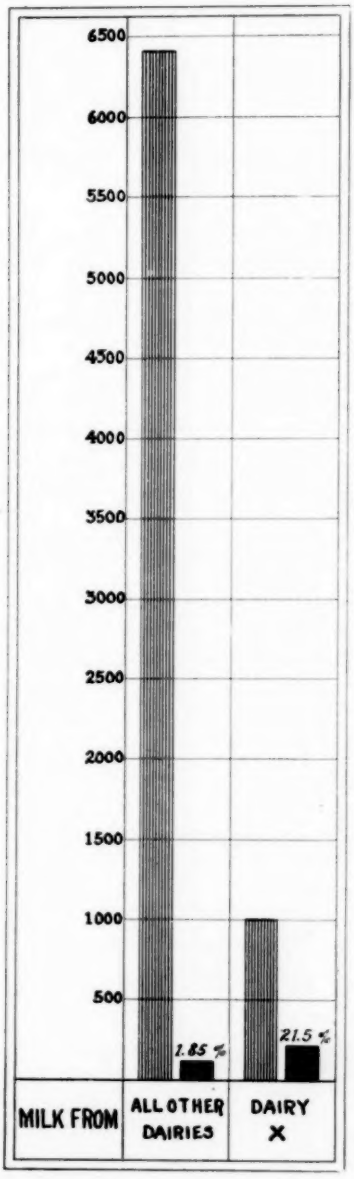

Chart 3.—Comparative morbidity of sore throat in the consumers of milk from Dairy X and from all other dairies.

In previous epidemics of septic sore throat the dairy investigation has been limited to those herds supplying milk that was under suspicion. Such a procedure fails to give information of the prevalence of garget in other herds as a basis of comparison. Furthermore, it is important to know if streptococcus mastitis may exist without being pathogenic to man. For these reasons the scope of dairy inspection was broadened so as to include all the cows in the larger dairies supplying the city.

On each farm it was ascertained if any members of the farmer's household had been afflicted with sore throat. The cows were examined carefully for caked bag or garget. Whenever garget was discovered, samples of the milk were taken separately in sterile tubes from each quarter of the bag under aseptic precautions, tightly sealed and forwarded at once to the Laboratory of Experimental Medicine, University of Illinois, Chicago.

In this manner inspection was made of 416 cows in thirty-eight different farms (Table 3).

Eleven cows were found with garget, which is about 2.6 per cent.

As the prevalence of garget in the Chicago epidemic was 4.6 per cent., we are not warranted in assuming that this disease was unusually common in the cows near Jacksonville. All of the cows with mastitis were placed under quarantine by the Milk Committee of the local medical society. Two of the eleven cases were caused by streptococci. These, it will be observed, came from farms that delivered milk to Dairy X.

In the farmers' families, six cases of sore throat were reported. None of them were milkers. All appeared after the outbreak of the epidemic. We think it is reasonable to assume, therefore, that these cases had no causal relation to the epidemic. Careful investigation, furthermore, failed to reveal any cases of sore throat about the dairies or distributing plants which could be brought into relation in any way with the outbreak of the epidemic. We thoroughly appreciate the importance of human "carriers" as possible sources for the infecting streptococci, but we could obtain no evidence that such persons played any rôle in the epidemic.

THE PLANT OF DAIRY X

Dairy X obtained its milk and cream from many farmers. The company sells milk and butter and a great deal of cream. It furnishes cream to the largest manufacturer and distributor of ice cream in the city. At the creamery plant is a pasteurizing apparatus which subjects the milk to a temperature of from 140 to 145 F. for a short and entirely inadequate period. *Cream was not pasteurized at all during the winter.*

TABLE 3.—Inspection of Cows and Dairies

Dairy	No. of Cows	Sore Throat on Farms	Infected Cows	Infecting Organism	Remarks
1	45	0	1	Staphylococcus albus	Cow hurt on wire fence.
2	9	0	1	Staphylococcus albus	Milk very yellow in color.
3	13	Girl—Dec. 15 2wks. duration	1 late in Nov.	Hemolytic streptococcus	Milk supplied to Dairy X.
4	13	0	1	Hemolytic streptococcus	Milk supplied to Dairy X.
5	4	0	1	Staph. albus and aureus Strep. viridans	
6	9	0	0		
7	28	0	0		
8	22	0	0		
9	11	0	0		
10	11	Boy—Jan. 26	0		
11	5	0	0		
12	6	0	0		
13	12	0	0		
14	2	2 boys—Jan. 14	0	.....	Use X Dairy milk
15	12	0	0		
16	6	0	0		
17	4	0	0		
18	14	0	1 (suspicious)	No organism found	
19	6	0	0		
20	5	0	0		
21	7	0	0		
22	6	0	0		
23	5	0	0		
24	7	0	0		
25	4	1 child—Jan. 6	0		
26	4	0	0		
27	9	0	0		
28	6	0	0		
29	13	0	0		
30	10	0	1—old case	Not examined	
31	7	0	0		
32	34	0	0		
33	15	1 case—Jan. 1-14 Dur. 10 days	0	.....	Milk to Dairy X.
34	17	0	0		
35	16	1 case—Dec. 25 Dur. 10 days	0	.....	Milk to Dairy X.
36	7	0	1—Nov. 24	Staphylococcus albus	Milk to Dairy X.
37	6	0	2—Jan. 1-14, Dec. 20-13	Staph. albus and aureus	
38	6	0	1	Staph. albus, fine bacillus	

None of the employees at the plant suffered from tonsillitis during the winter.

3. BACTERIOLOGICAL INVESTIGATION

The clinical symptoms, the general course of the disease, the character of the epidemic and its evident connection with the milk-supply would suggest at once that we have to do with a streptococcus infection, similar in character to the Chicago, Boston and Baltimore epidemics. Furthermore, the reports of throat examinations made by local physicians indicated the presence of numerous diplococci and streptococci in the smears obtained from the patients. Diphtheria was suspected often in these cases, but the cultures nearly always gave negative results for *Bacillus diphtheriae,* thereby showing that the infection was due to some other germ, probably streptococcus.

Owing to the fact that the epidemic was on the decline when the study began, not so many cultures from patients were obtained as we wished. In six typical cases, however, streptococci in large numbers were found as the predominating organism in smears and cultures of throat swabs. In one case an abscess occurred in the glands of the neck from which a pure growth of this organism resulted. The characteristics of these organisms isolated from throats will be considered later in connection with a discussion of the organisms obtained from the milk (Table 4).

As already stated, in an inspection of 416 cows from dairies about Jacksonville, eleven animals were found with diseased udders, and samples of milk were obtained from ten for bacteriologic examination. For this purpose the teats were cleansed and the first milk rejected. The milk was then collected directly in sterile test-tubes and immediately sent to the laboratory for examination. The results are presented in Table 3. Two cows were found having streptococcus mastitis. They were on different farms. The organisms were present in large numbers in the milk together with many leukocytes. The presence of the pus was immediately detected in the form of a slimy sediment in the centrifuge tube. A small amount of blood was present in one of the samples. In one cow two quarters were infected, in the other animal the four quarters were involved. Staphylococci were found in the milk from seven cows. In one was a fine, small, Gram-negative bacillus in large numbers and in another animal was obtained in smears and cultures an anhemolytic streptococcus similar in many respects to the *Streptococcus viridans,* but with no definite pathogenic properties for animals.

The properties of the streptococci isolated from the throats and from the diseased cows have been studied and the results are given in Table 4. Of the six strains isolated from the throats, four grow like

the streptococcus isolated during the Chicago epidemic two years ago.[1] The other two do not show these properties as distinctly and closely resemble the ordinary hemolytic streptococcus. The two strains isolated from the udders of the cows show capsules, a narrow zone of hemolysis, and are pathogenic for rabbits in doses of one to two slant agar tubes, producing multiple arthritis in from three to four days. They clearly belong to the *Streptococcus pyogenes* group and correspond to the epidemic streptococcus found in the throats. In Table 4 are given the properties of these various streptococci and their reactions on different sugars. The organisms from the cows and from the

TABLE 4.—Properties of Epidemic Streptococci from Throats and from Cows

No.	Origin of Culture	Hemolysis	Capsule	Pathogen. for Rabbits	Dextrose	Saccharose	Lactose	Mannite	Maltose	Inulin	Raffinose
1	Human 1 (Jacksonville)	+	+	+	+	+	+	0	+	0	0
2	Human 2 (Jacksonville)	+	+	+	+	+	+	0	+	0	0
3	Human 3 (Jacksonville)	+	+	+	+	+	+	0	+	0	0
4	Human 4 (Jacksonville)	+	+	+	+	+	+	+	+	0	0
5	Human 5 (Jacksonville)	+	0	+	+	+	0	0	+	0	0
6	Human 6 (Jacksonville)	+	+	+	+	+	+	0	+	0	0
7	Human 7 (Jacksonville)	+	0	+	+	+	+	0	+	0	0
8	Bovine 1 (Jacksonville) Dairy 3..	+	+	+	+	+	+	0	+	0	0
9	Bovine 2 (Jacksonville) Dairy 4..	+	+	+	+	+	+	0	+	0	0
10	Bovine 3 (Jacksonville) Dairy 5...	0	0	0	+	0	+	0	+	0	0
11	Epidemic Strep., human, Chicago epidemic	+	+	+	+	+	+	0	+	0	0
12	Epidemic Strep. Bovine, Chicago epidemic	+	+	+	+	+	+	0	+	0	0

throats agree in practically every detail. For comparison, Nos. 11 and 12 are typical streptococci isolated two years ago during the Chicago milk epidemic of sore throat. It will be seen that they do not differ essentially from the organisms isolated in the Jacksonville epidemic.

VIABILITY OF GERMS IN MILK PRODUCTS

During the study of this epidemic, questions were frequently asked as to the infectivity of sour milk, buttermilk, butter, cream, ice cream,

1. Davis: Bacteriologic Study of Streptococci in Milk in Relation to Epidemic Sore Throat, Jour. Am. Med. Assn., 1912, lviii, 1852.

etc. A somewhat detailed study of this problem is now being made, which will be reported in full later. Here only some general statements will be made. During the process of souring, hemolytic and epidemic cocci are gradually killed, as may readily be shown by making plates at regular intervals. These organisms are killed in three hours or less by the acidity of sour milk (forty-eight hours after curdling) and buttermilk. They die out in ordinary butter in the course of a few days. This is also due to the acidity, for in neutral butter they live for a long time. In ice cream streptococci remain alive for at least three weeks and without any appreciable diminution in number or virulence. Ice cream would seem to be the medium *par excellence* for the transmission and preservation of dangerous streptococci. Separator cream contains more organisms than the whole milk, and the skimmed milk contains less. The sediment contains large numbers. These facts are explained thus: The fat particles as they ascend carry with them mechanically a certain number of streptococci and other germs. On the other hand, many organisms are thrown down by centrifugation. In this way the number of organisms in the cream and the sediment is increased, while in the skimmed milk there is a decrease. Hemolytic and epidemic streptococci ordinarily do not multiply to any extent at 20 C. (68 F). At 27 C. (80.6 F.) there is little or no multiplication during the first six hours in milk (sterile or commercial), but at the end of twenty-four hours, in sterile litmus milk there is considerable multiplication. In ordinary commercial milk containing the common milk bacteria this multiplication is largely inhibited by the growth of these contaminating germs. Therefore, probably under few or no conditions met in the ordinary handling of milk can any appreciable multiplication of hemolytic or epidemic streptococci occur.

Even under most favorable temperature conditions multiplication of these streptococci in sterile milk does not begin for from two to three hours. It is, therefore, impossible for any appreciable increase of the dangerous streptococci to occur during the short time while the temperature of milk is being raised and lowered in the process of pasteurization.

SUMMARY

1. The epidemic of septic sore throat began abruptly, was most active in the first four weeks and gradually subsided after about eight weeks.

2. There were 348 cases of sore throat, most of them complicated by enlargement of the cervical glands and many by a marked exudate. Cultures from a number of throats showed a hemolytic streptococcus.

3. In a canvass of 913 households, in 60 per cent. of the cases of sore throats, milk or cream from Dairy X was used, although this dairy supplied only 17.5 per cent. of the households.

4. The morbidity rate in one institution supplied by Dairy X was 40 per cent.; in four other institutions supplied by other dairies it averaged 1.7 per cent.

5. Among 1,000 customers of Dairy X, 215, or 21.5 per cent., developed sore throat, whereas among 6,416 customers of other dairies, 133, or 2.1 per cent., developed the disease.

6. As a result of a systematic inspection of 416 cows on thirty-eight dairy farms, eleven cows with garget were found.

7. Bacteriological examinations revealed hemolytic streptococci in the milk of two of these diseased cows. The milk of both of these cows was distributed by Dairy X.

8. As Dairy X did not pasteurize the cream at all and the milk very inadequately, we may conclude that the streptococci originating in the bovine mastitis were probably responsible for the epidemic.

9. A study of these streptococci under varying conditions in the milk shows these characteristics:

(*a*) They do not appreciably multiply at room or warm temperatures because of the inhibiting influence of other milk bacteria.

(*b*) They are soon killed by the acidity of sour milk or buttermilk.

(*c*) They die out in ordinary butter in a few days, probably because of the acids present.

(*d*) They live in ice cream for at least three weeks without appreciable loss of numbers or virulence. Ice cream, therefore, is a peculiarly favorable medium for the dissemination of the germs.

10. Because of the extensive distribution of ice cream, manufactured from the Dairy X cream, we suspect that ice cream played an important rôle as a carrier in this epidemic.

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SPECIFIC HYPERLEUKOCYTOSIS

*STUDIES IN TYPHOID IMMUNIZATION. IV**

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We have already briefly described the specific and extreme grade of hyperleukocytosis that follows the intravenous injection of living typhoid bacilli in rabbits that have been immunized against this microorganism.[1] In the present communication we wish to consider more fully the detail and mechanism of this phenomenon and its analog in other types of protein immunization.

The importance of the polymorphonuclear leukocytes in both natural and acquired immunity against bacteria has been well and advisedly emphasized by Metchnikoff and his pupils.[2] The observations on the importance of leukocytes in immunity have dealt, however, with the qualitative rather than the quantitative response of these cells to the infecting agent. Our work, we believe, points out the existence and importance of a quantitative leukocyte response in cases in which recovery from an acute infection occurs abruptly.

The injection of apparently any foreign protein substance in normal animals may give rise to a hyperleukocytosis preceded or not by a leukopenia. Thus, Sacconaghi[3] found that subcutaneous injections of horse-serum in rabbits produces leukopenia followed by hyperleukocytosis. Simonds and Baldauf[4] found that the intraperitoneal injection of heated cultures of *Bacillus coli* or *B. pyocyaneus* in the peritoneal cavity of rabbits produces a rapid leukopenia in from twenty minutes to four hours followed by a hyperleukocytosis in two or three days of as high as 22,000, when the normal count was 12,000.

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* Submitted for publication May 6, 1914.

* From the Hearst Laboratory of Pathology and Bacteriology, University of California.

* We were assisted in this work by Mr. O. A. O. Sharpe, student in medicine.

1. Gay, F. P., and Claypole, E. J.: Specific and Extreme Hyperleukocytosis following the Injection of Bacillus Typhosus in Immunized Rabbits, Jour. Am. Med. Assn., 1913, lx, 1950; A Further Note on Specific Hyperleukocystosis in Immunized Animals, Proc. Soc. Exper. Biol. and Med., 1913, xi, 47.

2. Metchnikoff: L'immunité dans les maladies infectieuses, Paris, Masson & Cie, 1901.

3. Sacconaghi: Leucocitosi; organi; leucopoietici; immunita, Morgagni, Milan, 1905, xlvii, 282.

4. Simonds and Baldauf: The Relation of the Opsonic Index to the Leukopenia and the Leukocytosis Following Injections of Heated Bacterial Cultures, Jour. Infec. Dis., 1909, vi, 38.

Our first evidence of specific hyperleukocytosis was obtained, as is so often the case, in experiments designed for another purpose. Our method of testing the comparative value of different typhoid vaccines, the results of which are to be found in the following article,[5] has been to estimate the relative resistance of rabbits immunized by a given preparation against becoming "carriers." We are able to produce this carrier condition in rabbits with regularity on intravenous injection of one-half a standard blood-agar culture of a certain strain of *B. typhosus*.[6] Normal or insufficiently immunized animals become carriers, whereas the well-immunized animals give sterile cultures from the blood and gall-bladder within twenty-four hours. It was in the hope of tracing the fate of these myriads of injected bacteria in the circulation of the immune animals that we began making smears from the peripheral circulation soon after injection. To our surprise the organisms were not demonstrable in a smear made in ten or fifteen minutes after the injection; coincident, total and differential leukocyte-counts showed that the white blood-cells, particularly the polymorphonuclears, also diminished in a surprising manner, in many cases to one-sixth or one-eighth of their original number. Subsequent counts at rather irregular intervals soon showed that a leukocytic crisis occurs in from eighteen to twenty-four hours, and further counts showed that this crisis is preceded by another less marked one at about twelve hours. From the first, the grades of leukocytosis obtained in immunized animals have been controlled by parallel injections of the same amount of the same culture in normal rabbits, and in the latter animals there has been a surprising uniformity of counts. In the case of immunized animals it is far more difficult to be sure that the crest of the leukocytic wave has been attained, as the maximal count is of very short duration, as has been clearly determined in a few cases in which counts were made at half-four intervals. In other words, the rise of specific hyperleukocytosis is so abrupt that its height may well be missed unless the counts are frequent. The mean, therefore, of the immune counts as plotted in Figure 1 depresses rather than exaggerates the maxima individually obtained, whereas the normal counts better represent a true average (Fig. 1).

5. Gay, F. P., and Claypole, E. J.: An Experimental Study of Methods of Prophylactic Immunization Against Typhoid Fever. Studies in Typhoid Immunization V, THE ARCHIVES INT. MED., p. 671 this issue.

6. Gay, F. P., and Claypole, E. J.: The "Typhoid Carrier" State in Rabbits as a Method of Determining the Comparative Immunizing Value of Preparations of the Typhoid Bacillus. Studies in Typhoid Immunization, I, THE ARCHIVES INT. MED., 1913, xii, 613.

When the difficulties of counts for twenty-four hours at two-hour intervals are considered, it is not surprising that we have only seven counts in the immune animal and four in the normal that are fairly complete. Figure 1 is based on an average from counts in twenty-five immune and eight normal rabbits; the counts that were incomplete through the twenty-four-hour period were so divided in time as to fill the various hour intervals rather evenly.

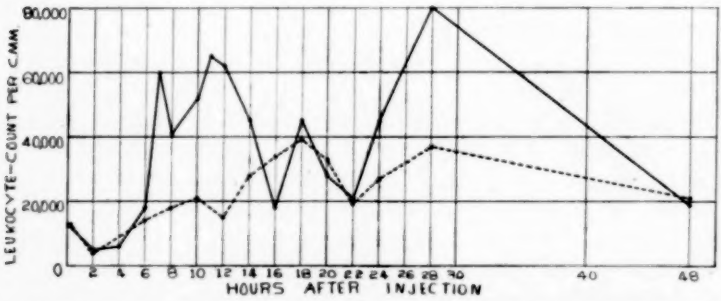
Some of the actual counts obtained are summarized in Table 1.

A differential count in either normal or immunized animals shows that both the leukopenia and hyperleukocytosis affects largely the polymorphonuclear type of cells. For example, in the counts of Immune Rabbit 108 given in Table 1, the differentials were as follows:

Before injection, total, 13,400: polymorphonuclears, 39 per cent.; small lymphocytes, 55 per cent.; large lymphocytes, 5 per cent.; eosinophils, 1 per cent.

Two hours after, total, 3,800: polymorphonuclears, 25 per cent.; small lymphocytes, 72 per cent.; large lymphocytes, 3 per cent.

Twenty-four hours after, total, 75,600: polymorphonuclears, 80 per cent.; small lymphocytes, 15 per cent.; large lymphocytes, 4 per cent.; eosinophils, 1 per cent.



Leukocytic response in normal and immune rabbits to injection of *Bacillus typhosus*. Immune rabbits are indicated by the solid line, normal rabbits by the broken line.

The abruptness of the leukocytic crisis in immune animals has already been mentioned; it may be illustrated by the following counts from Animal 65 on injecting the standard half blood-agar culture:

Leukocyte-count before injection, 13,800; two hours after injection, 6,400; eight hours after injection, 27,200; eleven hours after injection, 84,000; eleven and one-half hours after injection, 151,000; twelve hours after injection, 113,000.

That the exaggerated response in the typhoid-immune animals is specific is shown by an experiment in which a normal and an immune rabbit (typhoid) were given intravenous injections of one-tenth killed (60°) culture of *Micrococcus aureus*. The counts ran absolutely

TABLE 1.—Leukocyte-Counts in Normal and Immune Animals on Injecting One-Half Blood-Agar Culture Typhoid No. 38

Rabbit	Before	Hours After Injection											
		2	4	6	8	10	12	14	16	18	22	28	48
Normal 1	13,000	4,400	3,800	31,800	50,400	45,000	22,000	22,800	39,000	49,000	15,400	30,000	15,200
Normal 2	14,000	4,200	11,000	16,400	28,500	19,000	15,000	21,500	18,400	50,000	18,800	14,000	14,200
Normal 126	12,000	.....	.....	4,000	5,200	13,400	16,000	46,000	33,000	25,000	.....	.....	.....
Normal 127	14,200	.....	.....	6,400	5,700	6,600	15,000	20,000	34,000	33,000	.....	.....	.....
Immune 202	19,600	5,000	6,000	14,900	27,000	27,600	63,000	40,800	22,000	145,000	72,000	48,000	16,800
Immune 203	14,200	4,600	6,200	4,400	8,800	24,000	16,600	30,000	27,000	57,000	42,800	30,200	20,000
Immune 65	13,800	6,400	10,000	14,200	27,200	57,400	151,000	.....	.....	.....	.....	21,600	13,200
Immune 108	13,400	3,800	.....	33,600	85,600	.....	.....	.....	.....	.....	.....	25,000	10,400

parallel and showed little or no leukopenia and only a slight grade of leukocytosis in either animal.

A specific response in the typhoid-immune animals may be produced by the injection of various typhoid vaccines as well as by the living culture.

SPECIFIC HYPERLEUKOCYTOSIS IN OTHER TYPES OF IMMUNITY

Before considering more attentively the mechanism of this specific, quantitative reaction on the part of leukocytes, we may outline our experiments with the phenomenon in other types of immunity.

It can be shown that rabbits that have been immunized against red blood-cells of the sheep or guinea-pig react with a specific hyperleukocytosis to intravenous reinjection of the homologous cells. There is, of course, danger that animals so treated may die of anaphylactic

TABLE 2.—LEUKOCYTE RESPONSE OF RABBITS IMMUNIZED AGAINST—

Rabbit No.	Immunized Against	Hemolytic Titer	Reinjection
131	Sheep blood	.....	Sheep blood 1 c.c. intravenously
132	Sheep blood	.....	Sheep blood 1 c.c. intravenously
196	Sheep blood	1:4,000*	Sheep blood 1 c.c. intravenously
170	Control	.....	Sheep blood 1 c.c., intravenously
198	Control	.....	Sheep blood 1 c.c., intravenously
1	Guinea-pig blood	1:2,000†	Guinea-pig blood 1 c.c., 5 pct...
4	Control	.....	Guinea-pig blood 1 c.c., 5 pct...
2	Guinea-pig blood	1:2,000	Sheep blood 1 c.c., whole.....

* Determined with 1 c.c. of a 5 per cent. suspension of sheep blood and 0.1 c.c. guinea-pig
† Determined with 1 c.c. of a 5 per cent. suspension of guinea-pig blood and 0.2 c.c. rabbit

shock, but intraperitoneal reinjections designed to obviate this danger did not give very striking results. Some of the more striking examples of red blood-cell leukocytosis are summarized in Table 2.

It is evident from Table 2 that the phenomenon of specific hyperleukocytosis occurs with blood-cells as well as with bacteria, and the response would seem distinctly earlier. Our counts were designed simply to show that the phenomenon exists and are not so complete as in the case of the counts in the typhoid-immune rabbits; there is, in particular, no certainty that a second and higher crisis may not have been overlooked between ten and twenty-eight hours.

In view of Metchnikoff's findings of the phagocytic relations between macrophages (large mononuclear cells, endothelial cells and the like) and red blood-corpuscles, we had thought it possible that

this type of leukocytosis might bear some relation to a relative increase in this type of leukocytes. As far as our differential counts have gone, however, the same relative and absolute increase in polymorphonuclear cells as noted in the typhoid animals has been found.

In the case of serum immunity, we failed at first to catch the period of hyperleukocytosis, or else it failed in the first animals because they had not been sufficiently highly immunized. It was later clearly determined in two well-immunized animals by means of counts at short intervals (Table 3).

MECHANISM OF SPECIFIC HYPERLEUKOCYTOSIS

The well-known tropic action of immune serum as an adjuvant to phagocytosis suggested early in our studies that we might here be dealing with a similar phenomenon, the tropins in the circulation

—Red Blood-Cells to Their Respective and Other Corpuscles

	Leukocyte-Counts by Hours									
Before	1	2	3	4	5	7	8	9	10	28
18,400	.....	20,600	.....	33,000	34,500	47,800	41,000	42,000	.....	18 200
12,600	.....	22,800	.....	23,000	39,000	70,000	114,000	60,000	.....	12,400
10,000	.....	60 000	.....	61,000	41,000	27,300	15,200	.....	15,400	
5,660	.....	10,400	.....	9,600	8,800	9,200	6,500	.....	7,000	8 000
12,000	.....	24,000	.....	26,000	25,000	14,000	.....	.....	15,000	
12,500	15,600	64,000	126,000	9,000	38,000	12,400				
9,200	9,600	16,400	32,000	11,000	29,000	20,000				
8,000	16,200	13,200	15,600	9 200	14,000	17,000				

alexin.
alexin.

of the immune animal rendering the injected bacteria more attractive to leukocytes and, therefore, increasing their response. If this hypothesis were true, we should expect to be able to evoke a similar response in normal animals on the injection of sensitized or tropinized bacteria or red blood-cells. Such has proved to be the case as is shown by the following experiments:

Experiment 1.—A. To 1 c.c. of carefully washed sheep red blood-corpuscles was added 2 c.c. of a strong inactivated (56°) rabbit anti-sheep serum, and the mixture allowed to stand at 37 C. for one hour.

B. To the same amount of the same washed blood were added 2 c.c. of normal rabbit serum (56 C. or 104.8 F.) and the same contact allowed. The treated red blood-cells in both mixtures were then thoroughly washed three times in 0.85 per cent. sodium chlorid solution to remove serum. Two normal rabbits, Nos. 7 and 6, were given 1 c.c. intravenously of sensitized corpuscles

TABLE 3.—Leukocyte Response in Serum Rabbits Immunized Against Horse-Serum on Reinjection

Rabbit No.	Before	Leukocyte-Counts by Hours						
		1	2	3	3½	4	5	6
129.....	15,400	23,200	92,200	106,800	35,000	12,800		
128.....	10,200	6,100	10,600	.....	28,400	30,000	70,000	40,000
Control....	13,900	4,600	4,300	.....	5,700	6,600	5,900	.....

TABLE 4.—Leukocyte-Counts, Rabbits 7 and 6

Rabbit No.	Injection	Leukocyte-Counts by Hours*					
		Before	1	2	3	4	5
7	Sensitized blood..	.....	33,000	28,000	27,600	24,000	16,800
6	Unsensitized blood	14,000	70,000	194,000	28,000	12,000	.....

* Titer about 1:4,000.

TABLE 5.—Leukocyte-Counts by Hours, Normal Rabbit 10*

Before	1	2	3	4	5	6	7	8	9	10	11	12	13
10,400	9,000	5,400	9,000	4,200	6,800	9,600	6,600	11,200	12,600	26,400	20,800	130,000	19,000

* Agglutination titer, 1:4,000.

(*a*) and unsensitized corpuscles (*b*), respectively. Their leukocyte counts at following intervals are given in Table 4.

It is evident that the animal treated with sensitized blood showed a rapid rise of leukocytes to a maximum of 194,000 per cubic millimeter at two hours and then dropped rapidly to normal. The control treated with the same blood treated by a normal instead of an immune serum showed a nominal rise only. Similar, though not quite so striking, results have been obtained on injecting sensitized guinea-pig cells.

The phenomenon produced by injecting sensitized cells in normal animals may likewise be demonstrated on injecting sensitized bacteria.

Experiment 2.—A twenty-four-hour standard agar-culture of *B. typhosus* No. 5 was suspended in 2 c.c. of 0.85 per cent. sodium chlorid solution. To 1 c.c. of this suspension was added an excess of inactivated rabbit-antityphoid serum (1 c.c.). After contact for one hour at 37 C. (98.6 F.), the sensitized and clumped bacteria were washed free of serum by three washings in saline. (The effect of the same amount of untreated culture when injected intravenously in normal rabbits may be seen in Table 1).

The response of Normal Rabbit 10, given the sensitized culture, is given in Table 5.

This animal showed the characteristic high leukocyte-count of 130,000, twelve hours after injection, later than in the case of red blood-cells and corresponding to the rise obtained in immunized animals on injecting untreated bacteria.

THE POSSIBLE THERAPEUTIC SIGNIFICANCE OF AN ARTIFICIAL LEUKOCYTIC CRISIS

The work we have done with the specific leukocytic crisis, particularly the last fact, that it may be induced in normal animals by the use of sensitized (tropinized) cultures, immediately suggests therapeutic possibilities. Although our attention has hitherto been directed largely toward the prophylactic use of various typhoid vaccines, our preliminary experiments in animals in a curative way may be of suggestive value.

Metchnikoff has assumed that the principal locus of origin of the immune bodies is in the leukocytes. Our own experiments with determinations of the hemolytic and precipitin titer in blood- or serum-immunized animals, paralleling the leukocyte response on reinjecting the antigen (Tables 2 and 3), indicate that the crisis is followed by an increase in antibodies which rapidly restores the titer to as much or more than the original strength, which at first always suffers a temporary fall. In other words, the leukocytic crisis (and destruction?) would seem to liberate antibodies. We have already indicated that coincidently with the crisis in well-immunized typhoid animals the body sterilizes itself of the injected bacteria.

Similar processes are recognized as taking place in pneumonia, which is characterized by a crisis followed by rapid recovery. Dochez[7] has found that the protective value for mice of serums from human cases of pneumonia is increased after the crisis. Hektoen[8] also suggests the function of the leukocytic crisis in pneumonia as of curative value.

We have in a few instances been able to abort the usual carrier condition in rabbits that follows the injection of our standard dose of blood-agar culture of *B. typhosus* by injections of a sensitized typhoid vaccine, following the initial infection. Further study in this direction is at present engaging our attention.

Note in Conclusion.—In a recent article, Hamburger[9] has reported the leukocyte counts in children suffering from gonorrheal vulvovaginitis following intravenous injection of small amounts of a polyvalent gonococcus vaccine. A marked leukocytosis ranging from 15,000 to 28,000 was noted at the eighth hour, preceded by a hypoleukocytosis of from 6,000 to 7,000 at forty-five minutes. These results correspond to the results which we have obtained in immunized rabbits on reinjecting the antigen, although Hamburger has obtained only the first and less significant rise.

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7. Dochez: The Presence of Protective Substances in Human Serum During Lobar Pneumonia, Jour. Exper. Med., 1912, xvi, 665.

8. Hektoen: The Mechanism of Recovery in Pneumonia, Jour. Am. Med. Assn., 1914, lxii, 254.

9. Hamburger: Zur Vakzinebehandlung der kindlichen Gonorrhoe, Deutsch. med. Wchnschr., 1914, xl, 759.

AN EXPERIMENTAL STUDY OF METHODS OF PROPHYLACTIC IMMUNIZATION AGAINST TYPHOID FEVER

*STUDIES IN TYPHOID IMMUNIZATION. V**

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INTRODUCTION

Beumer and Peiper[1] were undoubtedly the first fully to appreciate the possibility of an active immunization against infection with the typhoid bacillus. In 1887 they were able to prove that mice that have recovered from a non-fatal infection with living typhoid bacilli are frequently protected against subsequent, larger, and usually fatal doses of the same organism. In their most successful experiment they found that the best results were obtained by the gradual increase in dosage on successive inoculations, and they further suggest that it may be possible to immunize by means of sterilized cultures, which, as had already been shown, contain the toxic principle of the typhoid bacillus. They raise the question as to whether it might not be possible to immunize human beings by means of gradually increasing amounts of such killed cultures. In the following year Chantemesse and Widal,[2] following the work of Salmon and Smith[3] on hog cholera, and of Roux and Chamberlain[4] on malignant edema, found that they could protect mice against infection with living typhoid bacilli by means of sterilized cultures of the organism.

The practical application of these experimental results in animals to the prevention of typhoid fever in human beings did not come until eight years later, following the discovery of the lysins by Pfeiffer. It was A. E. Wright,[5] who, in a preliminary publication in 1896 fol-

* Submitted for publication May 6, 1914.

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1. Beumer and Peiper: Bakteriologische Stüdien über die ätiologische Bedeutung der Typhusbazillen, Ztschr. f. Hyg., 1887, ii, 110.

2. Chantemesse and Widal: De l'immunité contre le virus de la fièvre typhoïde, conférée par des substances solubles, Ann. de l'Inst. Pasteur, 1888, ii, 54.

3. Salmon and Smith: On a New Method of Producing Immunity from Contagious Diseases, Proc. Biol. Soc. Washington, 1884-1886, iii, 29; Centralbl. f. Bakteriol., Orig., 1887, ii, 543.

4. Roux and Chamberlain: Immunité contre le septicémie conférée par des substances solubles, Ann. de l'Inst. Pasteur, 1887, i, 561.

5. Wright: On the Association of Serous Hemorrhages with Conditions of Defective Blood-Coagulability, Lancet, London, Sept. 19, 1906, ii, p. 802.

lowed by a fuller account by Wright and Semple[6] in the beginning of 1897, first outlined a method of immunizing human beings against typhoid fever. The method as outlined is notable on account not only of the essential facts involved, but also of the orderly and systematic method by which the problem was approached. Wright grew the culture of the typhoid bacillus in bouillon for two or three weeks and then killed them by heating to 63 C. (145.4 F.) for an hour, and preserved them with 0.5 per cent. phenol (carbolic acid). These vaccines were then tested for sterility and their toxicity carefully standardized by determining the minimal lethal dose for guinea-pigs; the dose chosen for injection in human beings was measured by this toxicity for animals. Wright further introduced a method of counting the number of the bacteria in the preparation employed by comparing their number in a given dilution when mixed with a suspension of red blood-cells, the number of which could be accurately determined. The dose of bacteria usually employed was from 750 to 1,000 million.

In the same year (1896) Pfeiffer and Kolle[7] described their method of immunizing and their method of estimating the protection that was produced in human beings by the agglutinins and the bactericidal potency of the serum. They employed agar-cultures of an avirulent strain of the typhoid bacillus suspended in salt solution and killed by heating to 56 C. (132.8 F.). An amount of this suspension, corresponding to one-tenth of an agar culture of something like 2 mg., was usually given on the initial injection, which was, as a rule, the only one. It is frankly admitted that the symptoms produced by this amount of culture were severe, and the method has since been modified in several ways to avoid these symptoms without essentially changing the principle involved.

So much for the first two communications on typhoid immunization in human beings. They form the groundwork on which subsequent methods of vaccination against typhoid fever have been built. The various methods that have since been advocated are numerous. Metchnikoff and Besredka[8] estimate that at least twenty different methods of vaccination have been described and advocated. Friedberger,[9] in his systematic review on typhoid immunization, enumer-

6. Wright and Semple: Remarks on Vaccination Against Typhoid Fever, Brit. Med. Jour., Jan. 30, 1897, i, p. 256.

7. Pfeiffer and Kolle: Experimentelle Untersuchungen zur Frage des Schutzimpfung des Mensches gegen Typhus abdominalis, Deutsch. med. Wchnschr., 1896, xxii, 735.

8. Metchnikoff and Besredka: Recherches sur la fièvre typhoïde expérimentale, Ann. de l'Inst. Pasteur, 1911, xxv, 193.

9. Friedberger: Die Methoden der Schutzimpfung gegen Typhus, etc. Kraus and Levaditi: Handbuch der Technik und Methodik der Immunitätsforschung, Fischer, 1908, i, 722.

ates twelve recognized methods. Paladino Blandini[10] has actually attempted to test the comparative immunizing value of seventeen preparations. It is not our purpose to describe all these methods in detail, and the reader who wishes further information on them may consult the systematic description of Friedberger[9] or of Fornet[11] in regard to them. It will be well, however, to outline the most important of these methods as evidence of the scope that the investigation has taken in perfecting this type of immunization, and as indicating the tendency which would seem to be leading to its gradual perfection.

PREPARATIONS OF THE TYPHOID BACILLUS THAT HAVE BEEN USED AS VACCINES

A. KILLED CULTURES OF THE TYPHOID BACILLUS

We have already mentioned that the first two preparations, those of Wright and of Pfeiffer and Kolle, consist essentially in killed cultures, the one, a bouillon-culture and the other a suspension of an agar-culture. These two original methods have been followed by many modifications. Thus Loeffler[12] took advantage of the fact that ferments, when dried, resist heating to a considerable degree without deterioration, and, regarding the antigenic property of the bacillus as ferment-like, dried suspended agar-cultures of the micro-organism and then heated them to from 120 to 150 C. (248 to 302 F.). These dried cultures were then pulverized and used in weighed amounts for immunizing animals. He states that such a culture has lost little of its property to produce antibodies. Friedberger and Moreschi[13] use a similar dried and heated culture, and administer it intravenously in very small doses; for example, an amount corresponding to 1/4,000 oese in immunizing human beings. It should be noted at this point that the method currently employed of determining the immunizing value of these preparations lies in an estimation of the antibodies (agglutinins, lysins, etc.) produced. As we shall later have cause to consider, these estimations offer an indication rather of the reaction of the animal body than a sure means of determining the degree of protection that has actually been afforded.

As we shall see in a moment, the use of living instead of dead cultures has been warmly advocated by certain observers, and their

10. Blandini, Paladino: Profilassi specifica del tifo addominale, Ann. d'ig. sper., 1905, xv, 295.

11. Fornet: Immunität bei Typhus; Kolle and Wassermann: Handbuch der pathologische Mikroorganismen, Ed. 2, Fischer, 1912, iii, 837.

12. Loeffler: Ueber ein neues Verfahren zur Gewinnung von Antikörpern, Deutsch. med. Wchnschr., 1904, xxx, 1913.

13. Friedberger and Moreschi: Vergleichende Untersuchungen über die aktive Immunisierung von Kannichen gegen Cholera und Typhus, Centralbl. f. Bakteriol., Orig., 1905, xxxix, 453.

assertions have apparently convinced several who are not quite willing to adopt such preparations owing to their possible danger, although they endeavor to approach them as far as possible without actually using living micro-organisms. It is apparently now the consensus of opinion that bacterial cultures are most antigenic when employed as nearly as possible in their living, unaltered condition, and that heat in particular tends to alter or destroy essential, characteristic, antigenic properties. Several methods have been advocated for avoiding or obviating so far as possible the destructive influence of heat, and at the same time killing the bacteria. Leishmann[14] advocates killing the typhoid bacillus at 53 C. (127.4 F.) instead of at 56 or 60 C. (132.8 or 140 F.). Vincent[15] while fully recognizing the superior value of living cultures, regards their use as dangerous, and therefore kills the vaccines that he employs by means of ether. As will be later mentioned, we have used alcohol for the combined purposes of killing the typhoid bacilli and accelerating their flocculation and drying. Levy and Bruch[16] killed their preparations by shaking the micro-organisms in a medium containing galactose, and find that organisms prepared in this way immunize guinea-pigs, as well as the living cultures, and that these two preparations are far superior to killed cultures in corresponding amounts. Fornet[11] regards the unpleasant effects that are produced by the heated vaccine as due not only to the heating itself but also to the presence of a large amount of albumin in the culture-medium. He therefore grows his micro-organism in a medium containing only a small amount of peptone and kills them by heating to 55 C. (131 F.) for fifty-five minutes. Courmont and Rochaix[17] killed their preparations by heating to 53 C. (127.4 F.), and further modified the usual method by administering the antigen through the rectum. Nicolle, Conor and Conseil[18] heat their bacteria to 55 C. (131 F.) for forty-five minutes, and then to 52 C. (125.6 F.) for thirty minutes more, and inject intravenously. Wassermann[19] insists that the antibodies produced by typhoid bacilli heated to 53 C.

14. Leishmann: Preliminary Note on Anti-Typhoid Vaccine in the Treatment of Enteric Fever, Jour. Royal Army Med. Corps, 1909, xii, 136.

15. Vincent: Sur la vaccination antityphique, Jour. State Med., 1912, xx, 322; Sur l'immunisation active de l'homme contre la fièvre typhoïde, Compt. rend. Acad. d. sc., 1912, clv, 480.

16. Levy and Bruch: Vergleichende experimentelle Untersuchungen zwischen drei Typhusvaccinen, die so wohl Bakterienleibersubstanzen als auch lösliche Stoffwechsel produkte enthalten, Arbeit. a. d. k. Gsndhtsamte, 1913, xliv, 150.

17. Courmont and Rochaix: Immunisation antityphique de l'homme par voie intestinale, Compt. rend. Acad. d sc., 1912, cliv, 611, 1829.

18. Nicolle, Connor and Conseil: De l'inoculation intraveineuse des bacilles typhiques morts à l'homme, Compt. rend. Acad. d. sc., 1912, clv, 1036.

19. Wassermann: Beiträge zur Typhus Schutzimpfung, Ztschr. f. Hyg., 1911, lxx, 204.

(127.4 F.) are not markedly better than when they are heated to 56 C. (132.8 F.). Renaud[20] has advocated the use of ultraviolet rays to kill the bacteria.

B. EXTRACTS OF BACTERIA

In addition to killed cultures of bacteria, numerous extracts and preparations derived from the bacteria have been advocated for the purpose of immunizing against typhoid fever. Hahn[21] has recommended the extract obtained from masses of bacteria by means of the Buchner press. McFadyen and Roland[22] have utilized liquid air as a means of killing bacteria and obtained from them an extractive substance. Neisser and Shiga[23] have utilized free receptors obtained by autolysis of bacteria at body temperature in salt solution. Wassermann[24] has suggested a similar method with the autolysis produced by distilled water. He subsequently dries the extract obtained in this way and uses it as a vaccine powder (*Impfpulver*). Brieger and Mayer[25] have used a watery, filtered extract of shaken bacteria. Bergell and Meyer[26] have used an extract of dried bacteria obtained by treating them with dilute hydrochloric acid.

Various so-called soluble toxins of the typhoid bacillus have also been suggested for immunizing purposes by Chantemesse,[27] by Werner,[28] and by Rodet, LaGriffoul and Wahby.[29] The extract of bacteria obtained by the method of Jez[30] has also been suggested.

C. LIVING CULTURES OF THE TYPHOID BACILLUS

Living cultures of the typhoid bacillus, usually more or less modified in their pathogenicity, have been warmly advocated by certain

20. Renaud: Vaccinothérapie par les vaccins irradiés (Etude biologique du vaccin typhique), Presse méd., 1911, xix, 655.

21. Hahn: Immunisierungs und Heilversuche mit plasmatischen Zellsäften von Bakterien, München. med. Wchnschr., 1897, xliv, 1347.

22. McFadyen and Roland: On the Intracellular Constituents of the Typhoid Bacillus, Centralbl. f. Bakteriol., Orig., 1903, xxxiv, 618.

23. Neisser and Shiga: Ueber freie Receptoren von Typhus und Dysenterie Bazillen und über Dysenterietoxin, Deutsch. med. Wchnschr., 1903, xxix, 61.

24. Wassermann: Zur aktiven Immunisierung des Menschens, Festschr. z. 60 Geburtst. v. Robert Koch, Fischer, Jena, 1904, p. 527.

25. Brieger and Mayer: Weitere Versuche zur Darstellung spezifischen Substanzen aus Bakterien, Deutsch. med. Wchnschr., 1903, xxix, 309.

26. Bergell and Meyer: Ueber eine neue Methode zur Herstellung von Bakterien Substanzen, welche zur Immunisierungszwecken geeignet sind, Med. Klin., 1906, xli, 753.

27. Chantemesse: Toxine typhoïde soluble et sérum antitoxique de le fièvre typhoïde, Progrès méd., 1898, iv, 16.

28. Werner: Sur la toxine excrété par le bacille typhique, Compt. rend. Soc. de biol., 1904, lvi, 836.

29. Rodet, Le Griffoul and Wahby: La toxine soluble du bacille d'Eberth, Compt. rend. Soc. de biol., 1904, lvi, 794.

30. Jez: Ueber Typhus Behandlung (Abdominal Typhus), mit einen antityphus Extract, Wien. med. Wchnschr., 1899, xii, 346.

observers as producing the best immunizing preparations in a manner similar to the methods that have been employed in dealing with other diseases, notably in cholera (Strong and Kolle). Castellani[31] uses an avirulent strain of the typhoid bacillus in the form of recent bouillon-cultures which are then partially killed by heating to 50 C. (122 F.) for one hour. Such a modified culture produces rather severe local and general symptoms, but when given twice would, to judge from Castellani's results, produce a most satisfactory degree of immunity, which apparently has lasted in a number of cases on which he reports for at least four years. He suggests, as an alternative, that the first injection may consist of a killed culture followed by a living culture on the second inoculation. In addition to the superior immunizing properties of the living culture, it is also pointed out by Fornet[11] that the killed cultures in a given dose give more reaction because the split products of proteins, which are recognized to be toxic, are liberated by heat. Living cultures have also been employed by Pescarolo and Quadrone.[32] The form of living cultures which has been advocated by Besredka will be considered under the next heading. As has already been mentioned, living cultures are generally admitted to be of superior immunizing value by many who are not willing to adopt them, owing to the real or fancied dangers coincident with their use, and this has led to an attempt to approach the condition of living bacteria without actually employing them. (Compare Vincent,[15] Levy and Bruch,[16] and particularly Metchnikoff and Besredka, to be considered presently.)

D. SENSITIZED CULTURES OF THE TYPHOID BACILLUS

The method of active immunization by means of sensitized vaccines, that is to say, by cultures that have been first treated with an immune serum and then killed, was introduced by Besredka[33] in 1902. This method is not infrequently referred to as serovaccination, but it differs from the method properly called serovaccination suggested by Leclainche[34] in swine erysipelas and by Calmette and Salimbeni[35] in plague, in that the excess of immune serum which these authors used is removed from the treated bacteria. It was found, as Besredka notes, that this excess serum tends to produce simply a passive immunity instead of the active immunity which is produced by the cultures treated with immune serum, and washed. Apart from his

31. Castellani: Typhoid and Paratyphoid Vaccination with Live Attenuated Vaccines, Lancet, London, 1912, i, 583; Observation on Typhoid Vaccination in Man with Attenuated Living Cultures, Centralbl. f. Bakteriol., 1909, lii, 92.

32. Pescarolo and Quadrone: Aktive Immunisation durch subcutane Injektion lebender Typhusbazillen bei Eberthschen Infection, Centralbl. f. inn. Med., 1908, xxix, 989.

original experimental work, Besredka did not deal with the practical aspects of sensitized vaccines until the experimental work on typhoid fever in apes was taken up by him in collaboration with Metchnikoff in 1911.[8] In the meantime, however, sensitized vaccines had been tried out apparently with considerable success in at least three instances. Marie[36] had been able to utilize the principle in treating rabies virus, Dopter[37] in vaccination against dysentery, and Theobald Smith[38] in a similar way found that he could produce active immunity by a balanced mixture of diphtheria toxin and antitoxin. This method of active immunization against diphtheria by a balanced (sensitized) mixture of toxin and antitoxin has recently been applied to human beings by von Behring.[39] The principal advantages of this method, as originally pointed out by Besredka and as apparently proved, are first, it produces little or no violent reaction on inoculation in instances in which the untreated bacteria themselves are distinctly irritating, as, for example, in plague. Secondly, it gives rise to an immediate though transitory passive immunity. Thirdly, it produces, eventually, an active immunity which is as enduring and as rapidly formed as when untreated bacteria are used.

Not a little experimental work was done with sensitized typhoid vaccine before the work of Metchnikoff and Besredka, which will be taken up later. Paladino Blandini[10] made a very careful study of seventeen different typhoid vaccines, comparing their relative immunizing properties. His experiments were carried out on guinea-pigs, which were treated by the various preparations and subsequently given an intraperitoneal dose of living typhoid bacilli. The vaccines tested include small doses of living cultures; killed cultures prepared after the method of Pfeiffer and Kolle, and of Wright; several "soluble toxin preparations" of the typhoid bacillus; nucleo-albumins; and extracts of the typhoid bacillus prepared in different manners. Compared with these methods was the sensitized vaccine employed by

33. Besredka: De l'immunisation active contre la peste, le choléra et l'infection typhique, Ann. de l'Inst. Pasteur, 1902, xvi, 918.

34. Leclainche: Sur la sérotherapie du rouget du porc, Compt. rend. Soc. de biol., 1897, xlix, 428.

35. Calmette and Salimbeni: La peste bubonique: Etude de l'épidémie d'Oporto en 1899, Ann. de l'Inst. Pasteur, 1899, xiii, 865.

36. Marie: Immunisation par des mélanges de virus rabique et de sérum antirabique, Compt. rend. Soc. de biol., 1902, liv, 1364.

37. Dopter: Vaccination préventive contre le dysentérie bacillaire, Ann. de l'Inst. Pasteur, 1909, xxiii, 677.

38. Smith, Theobald: Active Immunity Produced by So-Called Balanced or Neutral Mixtures of Diphtheria Toxin and Antitoxin, Jour. Exper. Med., 1909, xi, 241.

39. Von Behring: Ueber ein neues Diphtherie Schutzmittel, Deutsch. med. Wchnschr., 1913, xxxix, 873.

Besredka, and Blandini was able to demonstrate that the latter method was by all means the most protective. Not only were guinea-pigs protected for at least four months, but it was found that their serum, which contains sensitizers, also protects normal animals against infection. Ardin-Del-teil, Negre, and Raynaud[40] find that the use of sensitized typhoid vaccine in rabbits and human beings gives rise to relatively small amounts of agglutinins, but the bactericidal properties of the serum of those treated in this manner are much higher than of those treated by the ordinary cultures. These authors have also obtained very favorable results in treating cases of typhoid fever with this vaccine.

This failure of sensitized or agglutinated cultures to produce potent antibodies had already been noted by Neisser and Lubowski[41] and by Pfeiffer and Bessau.[42] Garbat and Meyer[43] immunized rabbits either with sensitized or with whole cultures and compared the properties of the serums of the two sets. The serum obtained by immunizing the rabbits with sensitized cultures agglutinated and gave the fixation reaction much less strongly than the corresponding animals treated with unsensitized cultures. The serums of the sensitized animals, however, were more bacteriotropic and protected animals experimentally much better than the serum of the animals treated with the plain vaccine. This fact is incidentally evidence of the unreliability of the agglutinin test as a measure of the grade of protection against typhoid infection, a matter which we shall later consider.

In 1911 Metchnikoff and Besredka[8] first reported their very important work on experimental typhoid fever in anthropoid apes. They found that the chimpanzee and the gibbon when given the dejecta from cases of typhoid fever, or lavishly contaminated food, or, in their latter experiments, when the mouth of the animal is painted with cultures of the typhoid bacillus, after eight days' incubation develop characteristic typhoid fever. Fifteen or sixteen monkeys that were tested gave positive blood-cultures on the tenth day, their serum contained agglutinins, and, in three instances, death due to the typhoid

40. Ardin-Del-teil, Negre and Raynaud: Sur la vaccinotherapie de la fièvre typhoïde, Compt. rend. Acad. d. sc., 1912, clv, 1179; Recherches sur les réactions humorales des maladies atteints par la fièvre typhoïde traités par le vaccin de Besredka, Compt. rend. Soc. de biol., 1913, lxxiv, 371; Récherches cliniques et expérimentales sur la vaccinothérapie de la fièvre typhoïde par le virus sensibilisé de Besredka, Ann. de l'Inst. Pasteur, 1913, xxvii, 644.

41. Neisser and Lubowski: Lässt sich durch Einspritzung von agglutinierten Typhusbazillen eine Agglutinproduktion hervorrufen? Centralbl. f. Bakteriol., Orig., 1901, xxx, 483.

42. Pfeiffer and Bessau: Zur Frage der Antiendotoxine bei Thyphus abdominalis, Centralbl. f. Bakteriol., Orig., 1910, lvi, 344.

43. Garbat and Meyer: Ueber Typhus Heilserum, Ztschr. f. exper. Path. u. Therap., 1910, viii, 1.

infection followed. The temperature of these animals ran as high as 40.8 C. (105.4 F.). Peyer's patches were found swollen but not ulcerated. The only detail in which this experimental disease would seem to differ from typhoid fever in human beings is the fact that the spleen in the chimpanzees is not distinctly enlarged. Having determined in this way that the typhoid bacillus is in reality the cause of typhoid fever and not some adherent filterable virus, as in the case of hog cholera, they turned their attention to methods of immunization against the disease. Their first series of experiments are far from convincing, although they draw from them rather sweeping conclusions. In the first place, the number of animals employed was necessarily small owing to their expense and the great susceptibility of the animals to extraneous infection, so that in this first series, in which there were five experiments, there was only one vaccinated animal in each case, tested with an untreated control. They come to the conclusion from their preliminary attempts that neither Vincent's typhoid vaccine, an autolysate of the typhoid bacillus, nor a *killed* culture sensitized according to the method of Besredka, will immunize anthropoid apes against a subsequent infection by the mouth.

Apart from the necessarily small number of animals in each experiment, certain other objections may be made to their experiments and conclusions, part of which Vincent[44] has pointed out. In the first place, the animals were tested very shortly after finishing the immunizing treatment, usually in from four to six days, which may reasonably be regarded as too brief a period for the establishment of the highest grade of immunity; and, secondly, the dose given the animals by the mouth, which is not stated very definitely, seems in all events to be extremely large, much larger, indeed, than in the natural infection in man. They conclude from their experiments that heated vaccines do not protect anthropoid apes against typhoid infection, and, owing to the analogy of the disease in these animals with the human syndrome, they regard such vaccines as unfitted to protect human beings. Inasmuch as the protective vaccination of man against typhoid fever by means of heated vaccines is generally recognized as reducing the morbidity to one-half or one-sixth of the normal, the logic of their position does not seem sound. It would seem better to have concluded that the immunization of the disease in anthropoid apes, as they have produced it, is not similar to their analogs in human beings.

In their second communication, Metchnikoff and Besredka[45] present experiments which, with the exception of one experiment in

44. Vincent: Remarques sur la vaccination antityphique, Ann. de l'Inst. Pasteur, 1911, xxv, 455.

45. Metchnikoff and Besredka: Des vaccinations antityphiques, Ann. de de l'Inst. Pasteur, 1911, xxv, 865.

which Vincent's vaccine is used, are more convincing. Their second series deals with the protective effect of vaccination by a living paratyphoid B culture, and by sensitized *living* cultures of the typhoid bacillus against subsequent infection of the latter organism in the manner described. These experiments comprise more animals than the previous series, and in addition the infection is not given until from ten to fifteen days after the completion of the treatment. Vincent's culture again failed to protect, whereas the living sensitized typhoid culture and the living paratyphoid B cultures uniformly saved the animals. It would be far from safe, however, to conclude from these experiments that no preparation of heated typhoid bacilli has the power to protect even anthropoid apes against subsequent infection by the typhoid bacillus.

We have examined the experimental details of the work of Metchnikoff and Besredka not in a spirit of unfavorable criticism, because, as will later be shown, we personally believe that their conclusions were in part correct, though the data on which they have based them have not convinced us.

We may here examine the results that have already been obtained in immunizing human being by the living sensitized cultures recommended by Metchnikoff and Besredka. Broughton Alcock,[46] working under their direction, was the first to report on the harmlessness of the method, its freedom from untoward effects and its apparent protective value. He advocates a dose corresponding to 1/100 of an agar-culture, which is equivalent to 500 million typhoid bacilli. Their organisms are sensitized by a few drops of a strong anti-typhoid serum, the exact potency and amount of which is not stated either by Metchnikoff and Besredka or by Broughton Alcock. After standing for twenty-four hours the clumped bacteria are washed in salt solution and resuspended in an aliquot portion of normal saline. A living sensitized vaccine prepared in this manner keeps for at least four months on ice. Objections have been raised by Vincent and others as to the danger of injecting living micro-organisms into the human body, but Metchnikoff and Besredka[47] have reported that careful tests fail to show that human beings treated in this manner become carriers of the typhoid bacillus or eliminate the micro-organism through the feces or urine. In view of the uniformly good results that have been obtained, there seems little reason further to suspect the harmfulness of the method. In a recent report Metchnikoff and

46. Alcock, Broughton: Vaccination for Typhoid by Living Sensibilized Typhoid Bacilli, Lancet, London, 1911, ii, 497.

47. Metchnikoff and Besredka: Sur la vaccination contre la fièvre typhoïde, Compt. rend. Acad. d. sc., 1912, clv, 112.

Besredka[48] have further endeavored to determine the optimal vaccinating dose and interval of this sensitized vaccine, and Besredka[49] reports some of the favorable results that have been obtained. Cadeau[50] vaccinated twenty-five patients, and only two showed slight general symptoms. In an asylum at Briqueville where many cases of typhoid fever existed in 1912, 516 persons were vaccinated, and in the twelve months subsequent, no cases occurred among them whereas four occurred in the 343 persons who were not immunized. Marie reports similar absence of general reaction. In all, Besredka has dispensed 10,000 doses of the vaccine and no unpleasant results have been reported except when the injections were given intramuscularly. He recommends a dosage of from 500 million to 1,000 million, although larger amounts could be given without harm. It is, of course, too early to draw any conclusive figures as to the actual protective value of this method, although its harmlessness and freedom from untoward symptoms seem to have been proved.

PERSONAL INVESTIGATIONS

In view of the general acceptance of the relative protective value of immunization with killed cultures of the typhoid bacillus, and the claim that this method of vaccination with sensitized living typhoid bacilli insures all the advantages of the more unpleasant method of treating with dead cultures, and protects as well or better, it would seem desirable to devise some method of animal experimentation which would enable us to settle conclusively this and many other debatable questions that arise in connection with this extremely beneficial process. There are still many questions in connection with typhoid vaccination which remain unsettled, such as the dosage to be employed, the interval at which the injection should be given, and most important of all, the duration of the protection that is afforded by any particular method and to any particular individual. Animal experimentation has, to be sure, been used for the most part on guinea-pigs that after treatment with various typhoid vaccines are infected in the peritoneal cavity. It seems justifiable, however, to question the transference of results obtained in preventing a banal peritonitis in this animal to human beings. The method of testing typhoid vaccines on anthropoid apes that Metchnikoff and Besredka have described is undoubtedly the ideal one, but practically impossible to employ for the settling of details of method owing to the expense of the animals and the difficulty in obtaining them in any numbers.

48. Metchnikoff and Besredka: Des vaccinations antityphiques, Ann. de l'Inst. Pasteur, 1913, xxvii, 597.

49. Besredka: Deux ans de vaccination antityphiques avec du virus sensibilisé vivant, Ann. de l'Inst. Pasteur, 1913, xxvii, 607.

50. Cadeau: Cited by Besredka (49), Ann. de l'Inst. Pasteur, 1913, xxvii, 607.

We have already described in some detail our method for testing the immunizing power of a given typhoid vaccine by means of the "rabbit-carrier" condition.[51] By employing one-half of a standard 10 per cent. blood-agar culture of a certain strain of *B. typhosus*, we have been able to produce this condition in normal rabbits with relative certainty. Thus of thirty control animals, twenty-eight, or 93 per cent., either became permanent carriers with positive cultures from blood or gall-bladder or, in a few instances, died acutely within twenty-four hours with symptoms of intoxication. For further discussion of the experimental advantages of this method over others proposed for this purpose, excepting experiments on anthropoid apes, we refer readers to our previous communication.

As we have just suggested, the presence of antibodies, particularly of agglutinins, is not a measure of the degree of protection against typhoid fever either in animals or in human beings. Antibodies indicate reaction which usually parallels protection but is by no means synonymous with it. In typhoid fever in particular the eventual absence of agglutinins in those recovered from and thereby protected from the disease indicates that serum tests are by no means to be confused with protection, which is more likely to be of a cellular than of a serum type. This indicates emphatically the necessity of testing the prophylactic immunizing value of any given vaccine by actual infection of the vaccinated animal rather than by any test of its antibodies. In our previous paper we have put forward our reasons for regarding the artificially produced bacteriemia in rabbits as analogous to typhoid fever in human beings, which with increasing agreement is now regarded primarily as a bacteriemia rather than a purely intestinal disease. The fact that we are able to prevent this carrier condition by previous immunization with one typhoid vaccine, whereas another preparation of the typhoid bacillus in the same amount does not prevent it, is further evidence that the method we have chosen is a delicate one and further suggests the variations in result that have been obtained in using different typhoid vaccines in human beings.

PREPARATION OF TYPHOID VACCINES FOR COMPARATIVE TESTS

The first essential in comparing various vaccine preparations seemed to us to lie in a standardization of the amount of substance employed. The methods usually employed in standardizing vaccines for clinical use may be accurate enough in view of the fact that the

51. Gay, F. P. and Claypole, E. J.: The "Typhoid Carrier" State in Rabbits as a Method of Determining the Comparative Immunizing Value of Preparations of the Typhoid Bacillus, Studies in Typhoid Immunization I, THE ARCHIVES INT. MED., 1913, xii, 613.

optimal dose is largely empirical and a little less or more may make no difference in the results. The fact that the usual methods of enumeration of bacteria by counting by the Wright method are being constantly changed is evidence enough that it is a far from satisfactory condition. We had already adopted the far more accurate method of dried, weighed bacterial preparations when the work of Wilson and Dickson[52] came to our notice. These authors have carefully determined the actual weights of the doses of various bacteria as commonly employed, checking their weighing results by counting the bacteria in a given volume of suspension. They find, for example, that there are on an average 8,000 million of typhoid bacilli in 1 mg. of a typhoid vaccine dried on platinum-foil. We were surprised to find how closely our estimations, although obtained in a different manner, agree not only among themselves, but also with the results of these authors. Our method of preparing dead typhoid vaccine has been to add normal saline (0.85 per cent.) in proportionate amounts to suspend forty-eight-hour cultures grown on Blake bottles containing 125 c.c. of 2 per cent. agar (titrated + 1) or, in certain experiments, of the same medium containing 10 per cent. fresh defibrinated rabbit blood. To such a suspension is added an equal volume of absolute alcohol, which kills the culture in the proportions used in less than fifteen minutes, flocculates out the organisms so that they can be easily collected by centrifugalization, and accelerates their drying in an unglazed porcelain plate in partial vacuum over sulphuric acid. Estimations of the number of typhoid bacilli in two different suspensions by counting (Wright's method) or by plating in successive dilutions, and by weight are summarized in the following experiments:

EXPERIMENT 1.—Vaccine 8: Nine Blake bottles containing 10 per cent. rabbit blood-agar were inoculated with a blood bouillon culture of *B. typhosus* 3b and incubated for forty-eight hours. The combined growths were suspended in a total of 220 c.c. of normal saline and shaken; 20 c.c. were kept for plating; 200 c.c. were precipitated with 200 c.c. of absolute alcohol, centrifugalized and dried *in vacuo* over sulphuric acid. The dried residue was ground for one hour in a mechanical ore-grinder (in a wooden case) sterilized by formaldehyd fumes and containing an agate mortar and pestle run by a small motor. This residue of microscopically disintegrated bacterial bodies weighed 675 mg.

By successive plates the bacteria in 1 c.c. of the original suspension were estimated to be 25,500 millions. In 200 c.c. the bacteria, weighing 675 mg., should equal 5,100,000 millions, or 7,555 millions to the milligram.

EXPERIMENT 2.—Vaccine 9: Nine Blake bottles of plain agar were inoculated with a bouillon culture of *B. typhosus* 3 and incubated for forty-eight hours. This growth was suspended in 200 c.c. of saline and the subsequent treatment was the same as in Experiment 1, except that the numerical estimation of the

52. Wilson and Dickson: Rapid Gravimetric Method of Standardizing Vaccines, Jour. Hyg., 1912, xii, 49.

living bacteria was made by comparison with red blood-cells in a stained preparation (Wright's method).

The weight of dried ground culture equaled 360 mg. We draw attention again[51] to the fact that the growth on blood-medium of *B. typhosus* is approximately twice that of the growth on agar. By count a cubic centimeter contained 13,000 millions, or 2,600,000 millions to 200 c.c., giving 7,222 millions to the cubic centimeter.

These two estimates of 7,555 millions and 7,222 millions of typhoid bacilli to the milligram may be compared with the 8,000 millions of Wilson and Dickson. Our interest in these figures, however, lies not in proving that the various methods of counting bacteria in our own and others' hands give surprisingly concordant results, but as giving us a comparison of weight to number so that one may state roughly in experimental and clinical work the weight dosage of the typhoid preparation required to give an amount comparable to, say, the classical 500 million bacteria ordinarily employed. There can be no question that in experimental work weighed amounts of dried preparations are more comparable and easier to determine than counted suspensions. Assuming the counts of Wilson and Dickson to be more accurate, the weighed amount of dried typhoid bacilli corresponding to a dosage of 500 million bacteria would be $\frac{1}{16}$ mg.

In all our experiments with vaccine preparations, then, we have used weighed amounts of dried and ground bacilli as a basis for comparison, except in those cases in which living cultures of the organisms have been employed, in which case comparisons were made in another way.

METHOD OF IMMUNIZATION

Having determined on the use of weighed amounts of dried, ground bacteria for comparative testing of typhoid vaccines, the next essential was to decide on the best method of immunization of the rabbits that are to be tested for protection against the carrier state.

Fornet and Müller[53] and Bonhoff and Tsuzuki[54] have already shown that for the production of certain antibodies an intensive method of immunization may be employed by reinjection at short intervals. One of us (Gay), in collaboration with Fitzgerald,[55] has still further perfected this method. We were able, for example, to produce high-grade hemolysins to sheep corpuscles by injecting rabbits intravenously on three successive days with 1 c.c. of washed

53. Fornet and Müller: Zur Herstellung und Verwendung praezipitierenden Sera, inbesondere für den Nachweis von Pferdefleisch, Ztschr. f. biol. Tech. u. Methodik, 1908, i, 201.

54. Bonhoff and Tsuzuki: Ueber die Schnellimmunisierungsmethode von Fornet und Müller, Ztschr. f. Immunitätsforschung, 1910, iv, 180.

55. Gay and Fitzgerald: An Improved Rapid Method of Producing Precipitins and Hemolysins, Univ. Cal. Pub. Path., 1912, ii, 77.

sheep blood. These lysins we find to be present in high degree four or five days after the last injection. Similar, although not quite so perfect, results were obtained in the production of precipitins and also in the production of bacterial agglutinins by Miss Edna Locke.[56] On the basis of these experiments, we had already determined that, provided a vaccine did not produce too severe symptoms, the period over which immunization extended might be markedly decreased without any corresponding deduction in the intensity of the immunity effected. It is of interest to note that Fornet[11] has also adopted a rapid method of immunization in vaccinating against typhoid. We have ourselves recommended, and Force[57] has employed immunization at intervals of two or three days in human beings, as will be mentioned later. The rapid method of immunization at short intervals not only may be shown in the case of rabbits to effect an equally high grade of immunity as determined either by antibody content or by actual resistance to infection, but also has the further advantage of actually producing less harmful effects when large amounts of the untreated vaccine are used. We have repeatedly noted that if a large total amount of vaccine is given to rabbits during a period extending over three weeks there is a larger percentage of mortality due to cachexia than when the same amount of vaccine is given within three days. The immunizing doses have invariably been three in number and have been administered either intravenously or subcutaneously; in all our later experiments it has seemed better to adopt the intravenous method as giving greater uniformity of results.

The advantage of immunization at short intervals as against longer intervals was first tested by comparing the resistance to the carrier state of two sets of rabbits, the one immunized by three intravenous injections on three successive days, the other by three equal doses administered intravenously at three-day intervals. There was found to be no marked difference in relative resistance of these two sets of animals when given the test dose two months later. The agglutinins averaged the same titer in the two sets. In a more systematic experiment, a comparison of three daily with three five-day interval doses was made (Experiment 3).

EXPERIMENT 3.—Fifteen rabbits weighing from 2,000 to 2,500 gm. each were divided into two lots of eight and seven animals and immunized as follows:

Lot 1: Eight animals were given three doses of ⅙ mg. of dried, sensitized[58] Vaccine 13 intravenously in a volume of 1 c.c. 0.5. per cent. carbolated saline

56. Locke, Edna: A Rapid Method of Producing Bacterial Agglutinins, Univ. Cal. Pub. Path., 1912, ii, 91.

57. Force: Institutional Vaccination Against Typhoid Fever, Am. Jour. Pub. Health, 1913, iii, 750.

58. Gay, F. P., and Claypole, E. J.: The Typhoid "Carrier-State" in Rabbits

solution, Oct. 6, 13 and 22, 1913, that is, a total of 0.5 mg. in a period of sixteen days.

Lot 2: Seven animals were given three doses of ⅙ mg. of dried, sensitized Vaccine 13 intravenously, Oct. 20, 21 and 22, in a volume of 1 c.c. 0.5 per cent. carbolated saline solution, that is, a total of 0.5 mg. in a period of three days.

Before test inoculation, in Lot 1, three animals died with extreme emaciation and no distinctive lesions (cachexia)—one of an intercurrent infection and one of injury. That is, in four of the eight animals death may reasonably be attributed to the vaccine. No animals in Lot 2 died during this period.

On the forty-fourth day after the end of immunization, the survivors of Lot 1, and those of Lot 2 were given each one-half standard blood-agar culture of *B. typhosus* 3b intravenously. Necropsies were performed on the animals that died, cultures were made from the blood and bile. The survivors were chloroformed, and cultures made from blood and bile on the ninth day. When organisms of proper morphology and cultural characteristics were isolated from either source they were tested by agglutination with a strong antiblood typhoid serum.[58] The results of this experiment may be summarized in Table 1.

TABLE 1.—RESULTS OF LONG AND SHORT-INTERVAL METHODS OF TYPHOID IMMUNIZATION IN RABBITS

	Died During Vaccination	Carriers	Recovered
Short intervals	0	3	4
Long intervals	4	3	0

This experiment as well as other similar ones would seem to indicate the superior value of the short-interval intensive method of immunization.

DOSAGE OF VACCINE PREPARATIONS

We have no contribution to make on the optimal dose of typhoid vaccine for prophylactic use in human beings. As will be seen later, our choice for such purposes has been largely empirical and has followed accepted usage. In our comparative experimental work, however, it has been necessary not only to use preparations derived from weighed, dried bacteria, but also to determine with increasing accuracy the duration of the protection afforded by a given amount of a standard preparation. In our first experiments we immunized separate series of rabbits each with a given preparation and then tested representatives of each series at several successive periods. Inasmuch as we started with rather large doses of vaccine, the time before the protection wore off was rather long and the method time-consuming.

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as a Method of Determining the Comparative Immunizing Value of Preparations of the Typhoid Bacillus. Studies in Typhoid Immunization I. Agglutinability of Blood and Agar Strains of the Typhoid Bacillus, Studies in Typhoid Immunization II, THE ARCHIVES INT. MED., 1913, xii, 621. See later, Experiment 4.

By decreasing the total vaccinating dose we were finally able to estimate with certainty that a given amount of one type of vaccine would protect for five or six weeks; with this point determined, it was easy to compare the immunizing value of other preparations with this one. In nearly all experiments, two or more control normal animals have been injected, although their use is not absolutely imperative since the less well protected animals become carriers and the percentage of carriers in each series gives the basis of comparison.

DETERMINATION OF THE TYPHOID VACCINE OF ELECTION FOR IMMUNIZATION

The carrier condition was at first determined in the immunized and control animals by cultures taken from the blood ten and twenty days after inoculation; the results were checked by the post-mortem condition and cultures from the bile when the animals died, or in the case of survivors by chloroforming them from fifty to seventy days later. A later method which gives quicker results is to chloroform all the survivors at the end of ten or fourteen days and make cultures from the blood and bile. A perfectly immunized animal is one that gives negative blood-cultures and at death presents no lesions particularly of the gall-bladder and gives negative cultures from the bile.

Throughout our experiments it has been our effort to kill our cultures of *B. typhosus* in a manner designed to obviate, so far as possible, the deteriorating effect of heat that has been evidenced by the work of numerous observers mentioned before. The introduction of heat, at all events for the purpose of obtaining sterile cultures, seems to us quite unnecessary, and, as one of us pointed out a number of years ago,[59] cultures of the dysentery bacillus may be sterilized and preserved by the simple addition of 0.5 per cent. trikresol or phenol, without destroying their toxic and antigenic properties to the same degree as in corresponding cultures that are first killed by heat at 60 C. (140 F.) and then preserved by trikresol. Very little attention appears to have been paid to this observation, although we believe that several have utilized the method with advantage, killing the cultures either with liquor formaldehydi or with trikresol. In the present series of experiments we have desired not only to kill the bacteria with little harm but also to collect and dry them in order that the dosage employed might be reckoned from weighed amounts of the dried bacilli, which had been previously ground in an agate mortar. For this purpose, as already stated in Experiment 1, we

59. Gay: Vaccination and Serum Therapy Against the Bacillus of Dysentery, Univ. Penn. Med. Bull., 1902, xv, 307.

precipitate our various bacterial suspensions with equal parts of absolute alcohol, which accelerates the centrifugalization and the drying of the preparation. If the culture is to be sensitized, a definite amount of a potent, immune serum from the rabbit is previously added, the dosage being estimated in accordance with the agglutination titer of the serum in question and the number of bottles from which it was obtained, the amount being 1 c.c. of a serum that agglutinates the organism in question in a dilution of 1:20,000 to the suspended growth from each of the seven Blake bottles. After standing for two or three hours in an incubator at 37 C. (98.6 F.), the sensitized culture is left in the ice-chest over night, centrifugalized, the supernatant fluid removed, the sediment washed in the original amount of normal saline, recentrifugalized, and the volume restored to normal with sterile saline. This washed sensitized culture is then flocculated by adding an equal volume of absolute alcohol, dried, ground and weighed as in the unsensitized culture.

TABLE 2.—SUMMARY OF EXPERIMENT 4

Rabbit No.	Vaccination	Interval Before Inoculation Weeks	Agglutinins	Result
9	Plain Vaccine 1	12	1:100	Recovered
10	Plain Vaccine 1	12	Neg. 1:100	Carrier
12	Plain Vaccine 1	12	1:500	Carrier
13	Sensitized No. 1	12	Neg. 1:100	Recovered
14	Sensitized No. 1	12	Neg. 1:100	Recovered
Control 71	.....	..	.....	Carrier
Control 72	.....	..	.....	Carrier

We first compared the protective value of sensitized and unsensitized cultures. The results of our first experiment (Experiment 4) may be given, although few animals are involved, because the results were particularly clear-cut. Similar results will be shown in a later experiment in connection with other preparations.

EXPERIMENT 4.—Dried sensitized and unsensitized vaccines were prepared from the same growth of *B. typhosus* 5 (Army and Navy, Dorset strain) in the manner described above. Four rabbits were given 0.56 mg. of the dried unsensitized vaccine intravenously in 1 c.c. carbolated saline solution for three successive days. Two other rabbits were given the same amounts of the same vaccine *sensitized* in the same manner. One of the first series (plain vaccine) died by accidental injury. Three months after the last injection, the agglutination titer of the immunized rabbits was tested and they and two control animals of similar weights were given the standard test dose of living blood-agar typhoid bacilli (½ culture *B. typhosus* 3c) intravenously. The results are summarized in Table 2.

This experiment indicates the superior value of a sensitized vaccine over an unsensitized vaccine in producing a durable immunity. It further shows the unreliability of agglutinins as indicating resistance to actual infection with the typhoid bacillus. As we have already noted in the introduction, other observers have found that sensitized bacteria produce less agglutinins than untreated bacteria, but more tropic or sensitizing antibodies.

The method which we have described for preparing and grinding the vaccine is essentially similar to the one described by Besredka for the preparation of endotoxins, and it may, indeed, be shown in the case of the untreated vaccine that the supernatant fluid contains the greater part of the toxic substances of the whole vaccine (Besredka,[60] Steinitzer[61]). It seemed to us important to determine at this point whether or not the supernatant fluid of such a ground and suspended preparation of typhoid bacilli, or the sediment of bacterial bodies left on centrifugalizing this mixture contains the immunizing principle. In the following experiment (Experiment 5), in which the immunizing value of the two preparations is shown, it is very evident that it is the sediment or bacterial bodies deprived to a great extent of toxicity of the whole vaccine which is the essential immunizing principle. This immediately suggests the advisability, from the point of view of symptoms produced and of protecting value assured, of utilizing simply the remnants of the bacterial bodies obtained in such a vaccine rather than the whole vaccine itself. Experiments designed to test out the comparative value of the whole vaccine, on the one hand, and the sediment on the other, show very distinctly that the sediment possesses not merely as much but apparently actually more immunizing value than the whole vaccine (Experiment 6).

EXPERIMENT 5.—In this experiment are combined the results obtained from two different plain unsensitized vaccines, Vaccine 1, which has already been described (Experiment 4), and Vaccine 8 prepared in the same way but from blood-agar cultures grown on a blood-agar medium. The weighed, dried vaccine was in each case suspended in the proper amount of 0.5 per cent. carbolated saline solution and allowed to stand for forty-eight hours with thorough shaking at intervals. The suspended vaccine was then centrifugalized thoroughly, leaving an opalescent *supernatant* fluid (endotoxin) and a grayish white *sediment* of ground bacterial bodies. The supernatant fluid was removed and the sediment restored to the original volume by adding fresh carbolated saline solution. Rabbits were then vaccinated in series with the supernatant and sediment, respectively. The dosage of vaccine preparations given in three doses on successive days (volume 1 c.c. on each injection) and the resultant

60. Besredka: Etudes sur le bacille typhique et le bacille de la peste, Ann. de l'Inst. Pasteur, 1905, xix, 477.

61. Stenitzer: Ueber die Toxine (Endotoxine) der Typhusbazillen; Kraus and Levaditi, Handbuch der Immunitätsforschung, 1908, i, 193.

condition obtained on testing the animals with the standard dose of living culture is summarized in Table 3.

Table 3 shows the marked superiority of sediment over supernatant fluid in producing immunity from infection; all of the six sediment-treated animals recovered, whereas only one of eight supernatant animals failed to become carriers. The further unreliability of the agglutinin test is shown by the fact that in the first series the supernatant gives higher agglutinins but no protection.

TABLE 3.—COMPARISON OF IMMUNIZING POWER OF THE SUPERNATANT AND SEDIMENT DERIVED FROM PLAIN TYPHOID VACCINES*

Rabbit No.	Vaccination Employed	Interval Before Testing Months	Agglutinins	Result
21	Supernatant of Vaccine 1. Total dosage, 1.68 mg. in three days	2	1: 50	Carrier
22	.....	2	1: 400	Carrier
26	.....	2	1: 200	Carrier
27	.....	2	1: 500	Carrier
23	Sediment of Vaccine 1; 1.68 mg. in three days	2	1: 100	Recovered
28	.....	2	1: 400	Recovered
29	.....	2	1: 100	Recovered
		Weeks		
85	Supernatant of Vaccine 8; 10 mg. in three days	6	.....	Carrier
86	.....	6	.....	Recovered
87	.....	6	.....	Carrier
88	.....	6	.....	Carrier
73	Sediment of Vaccine 8; 10 mg. in three days	6	.....	Recovered
75	.....	6	.....	Recovered
76	.....	6	.....	Recovered

*The control rabbits are purposely omitted for simplification; they were positive, as is the rule.

One of our most recent experiments summarizes the facts that have been adduced as to the most protective preparation, and further brings out the fact, already stated, that the sediment actually protects better than the whole vaccine. In comparing the whole suspended vaccine with the sediment derived from such a vaccine, it should be noted that every technical error necessary to produce the sediment

from the whole vaccine would tend to lessen the amount of material in the sediment, and the superior immunizing value of the latter is therefore so much the more striking. The results of this experiment are summarized in Table 4. The preparations used were prepared as already described, the preparations all being derived from an original suspension of *B. typhosus* 4 (recently isolated stain, October, 1913), grown on agar (Vaccine 20).

TABLE 4.—Comparison of Whole Suspended Vaccine with Sediment*

Rabbit No.	Vaccination Employed	Interval Before Testing Days	Result
262	0.5 mg. whole unsensitized Vaccine 20 in three days intravenously	32	Carrier
265	.....	32	Recovered
266	.....	32	Carrier
263	.....	32	Died before testing
264	.....	32	Died before testing
267	0.5 mg. sediment unsensitized Vaccine 20 in three days intravenously	32	Carrier
268	.....	32	Recovered
269	.....	32	Carrier
270	.....	32	Recovered
271	.....	32	Carrier
273	0.5 mg. whole sensitized Vaccine 20 in three days intravenously	32	Died acutely (anaphylaxis)
274	.....	32	Killed by injury
275	.....	32	Recovered
276	.....	32	Recovered
277	0.5 mg. sediment sensitized Vaccine 20 in three days intravenously	32	Carrier (bile culture only positive)
278	.....	32	Recovered
279	.....	32	Recovered
280	.....	32	Killed by accidental injury
281	.....	32	Recovered

* Controls again purposely omitted.

The points which we have already brought out are emphasized in this experiment. Thus the percentages of recovery are 33 for whole unsensitized, 60 for whole unsensitized sediment, 66 for whole sensitized, and 75 for sensitized sediment. The first series also shows the greater toxicity of the whole culture as compared with its sediment.

We seem so far to have determined, then, that killed, sensitized cultures are somewhat more protective than plain, unsensitized, killed cultures of the typhoid bacillus and that the protection is due to the bacillary sediment and not to the supernatant endotoxic fluid. These sediments, moreover, seemed distinctly more protective than the whole vaccines from which they were derived.

In view of the assertions of Metchnikoff and Besredka, it remains to determine by our method whether or not living, sensitized cultures have any superior immunizing value over sensitized cultures killed

TABLE 5.—COMPARISON OF LIVING SENSITIZED WITH DRIED WHOLE SENSITIZED VACCINES

Rabbit No.	Vaccination Treatment	Interval Weeks	Result
171	1 mg. living sensitized culture* intravenously in three doses, on three successive days.	7	Recovered
172	.....	7	Carrier
173	.....	7	Carrier
174	.....	7	Carrier
175	.....	10	Recovered
176	.....	10	Recovered
179	1 mg. dead sensitized culture intravenously in three doses, on three successive days.	7	Carrier
180	.....	7	Recovered
181	.....	7	Carrier
182	.....	7	Carrier
184	.....	10	Carrier
185	.....	10	Recovered
186	.....	10	Recovered

*Computed from same culture dried.

by alcohol. For this purpose we have compared the living sensitized culture first with whole dried sensitized culture and then the living sensitized culture with the sensitized sediment. In these experiments the original bacterial suspension is sensitized, washed, and divided into two parts, one of which is left intact and the other precipitated by alcohol, dried, ground, weighed, and suspended in the original amount of fluid. Here again technical errors would militate against the immunizing strength of the dried culture, particularly when the latter is carried further to the sediment preparation. The results of two such experiments are summarized in Tables 5 and 6.

The first experiment shows that there is little or no difference to be shown between the immunizing powers of living sensitized cultures

as compared with sensitized cultures killed by alcohol. The second experiment shows the immunization by dead sensitized sediment to be distinctly superior to that by living sensitized cultures.

PROTECTIVE VACCINATION IN HUMAN BEINGS BY MEANS OF THE MODIFIED SENSITIZED CULTURE

Our experimental results had reached a stage nearly a year ago when we felt justified in recommending the whole sensitized culture that we have described for use in protecting human beings against

TABLE 6.—COMPARISON OF LIVING SENSITIZED CULTURE WITH DEAD SENSITIZED SEDIMENT *

Rabbit No.	Vaccination Treatment	Interval Weeks	Result	
296	1 mg. living sensitized culture intravenously in three doses on three successive days.	†		
289	.....	7	Carrier	A
290	.....	7	Carrier	
291	.....	7	Recovered	
292	.....	7	Carrier	
295	.....	7	Carrier	
293	.....	8	Carrier	
297	.....	10	Recovered	
305	1 mg. dead sensitized sediment intravenously in three doses on three successive days.	†		
299	.....	7	Recovered	B
300	.....	7	Recovered	
302	.....	7	Carrier	
303	.....	7	Recovered	
304	.....	7	Recovered	
298	.....	8	Carrier	
301	.....	9	Carrier	
306	.....	9	Recovered	

* Of group A, two animals, or 25 per cent., recovered, and six animals, or 75 per cent., remained carriers. Of group B, five animals, or 62.5 per cent., recovered, and 3 animals, or 37.5 per cent., remained carriers.
† Died before testing.

typhoid fever. It seemed demonstrated from the work on rabbits that the sensitized vaccine, washed, precipitated, killed with alcohol, and ground would protect at least as well and probably better than the ordinary vaccine that had not been sensitized, and we anticipated much fewer untoward symptoms following vaccination by the use of this sensitized vaccine. This absence of untoward effects has been fully justified in experience. Up to the present time over 1,000

students in the University of California have been immunized by Professor Force of the Department of Hygiene. The absence of reaction or the mildness of reaction has been very striking. Force[57] has already reported on the first 261 cases treated by this method, and less complete analysis of the subsequent cases agrees with the results obtained in the smaller number. In these 261 cases — apart from the local symptoms of induration, redness, and slight tenderness for one or two days—general symptoms, even of a mild grade, were very seldom observed. The vaccine was administered on alternate days, the treatment being completed within a week. The very fact that it was possible to give the second and third vaccinations on the second and fourth days after the first indicates strongly that even the local condition was so mild as to allow the subsequent inoculations. The dosage at first employed was $\frac{1}{16}$ mg. of dried culture suspended in 0.5 per cent. carbolated salt solution, corresponding, as we have determined, to 500 million bacteria. Since this produced little or no reaction, we have since increased it by one-half ($\frac{3}{32}$ mg.). In only six cases in the total number of inoculations practiced (671) did the temperature rise above 38 C. (about 100 F.). The symptoms were somewhat more pronounced in women than in men. In two cases of arrested tuberculosis the reaction was more marked and in a few cases that gave a history of previous typhoid fever the reaction was also slightly more pronounced. More recently we have employed the sensitized sediment vaccine in accordance with the experimental results that have just been obtained, which show conclusively that this substance is more immunizing than the whole sensitized preparation. This sensitized sediment gives even fewer reactions than the whole sensitized vaccine. Thus in a recent series that Force has vaccinated with sensitized sediment, using a dosage corresponding to 750 million bacteria, including 672 separate injections, malaise was noted in 1.8 per cent.; fever (above 99 F.) in 4.5 per cent.; pain in back in 2.4 per cent.; pain in head in 9 per cent., and pain in the arm in 3 per cent. In cards returned to us from 231 outside patients to whom the vaccine was distributed, 215 report total absence of local and general reactions (93 per cent.). Ten cases were marked as having shown generalized symptoms and three cases of typhoid recoveries and three patients who had been previously vaccinated also showed slight symptoms. These reactions may be compared with the following: Hartsock[62] obtained a mild reaction, by which he specifies "temperature up to 100 F. with slight general reaction, malaise, and considerable local tenderness," in 83 per cent. of men treated with U. S.

62. Hartsock, F. M.: Antityphoid Vaccination, Jour. Am. Med. Assn., 1910, liv, 2123.

Army vaccine. Hatchel and Stoner[63] in 1,326 cases vaccinated with a polyvalent vaccine found malaise in over 58 per cent. and fever in 43 per cent. Albert and Mendenhall[64] used the U. S. Army strain in regular doses and intervals and found that the local reaction lasted for from three to five days. Fever as high as 103 F. was usual and malaise, headache, and insomnia were common.

Auché and Chevalier[65] have reported a notable failure of prophylactic typhoid vaccination in a person who received three injections in January, and in July came down with a well-defined case of typhoid fever. We agree with the authors that such cases should be reported as indicating the limitations of the method. Fornet has collected further cases in which the protection was not produced. In our early series of cases among the students treated with whole monovalent sensitized vaccine, in which it has been possible to follow the results with great accuracy, there has been one case of typhoid fever among the vaccinated persons five months after treatment; the recovery was uneventful. These instances go to prove that further perfecting of any method in vogue is necessary. Both Wassermann[24] and Vincent[15] have suggested the use of polyvalent vaccines; although the typhoid bacillus has been regarded as an unusually fixed species, the occurrence of minor biologic peculiarities, particularly in the fermentation of sugars, has been noted in the collection of organisms which we have studied. In accordance with these facts, as well as with the results that we have detailed, we have recommended a polyvalent sensitized sediment for prophylactic immunization against typhoid fever in human beings. The organisms, five in number, which have been used to produce the polyvalent mixture was isolated from recent cases of typhoid fever in the vicinity. An additional advantage in the use of sensitized over unsensitized cultures is that a strain of the typhoid bacillus may be used as in our polyvalent vaccine without producing any more symptoms than with a monovalent vaccine. It is particularly unnecessary to seek a "mild strain" as in the case of the U. S. Army vaccine. Our various strains are grown separately on Blake flasks, and the suspended cultures mixed and treated with a polyvalent immune serum obtained by immunizing rabbits with each of the strains in turn. This polyvalent sensitized sediment vaccine is now being manufactured and distributed free of cost by the California State Board of Health to any physicians in the state who may apply.

63. Hatchel, F. W., and Stoner, H. W.: Inoculation Against Typhoid, Jour. Am. Med. Assn., 1912, lix, 1364.

64. Albert and Mendenhall: Reactions Induced by Antityphoid Vaccination, Am. Jour. Med. Sc., 1912, cxliii, 232.

65. Auché and Chevalier: Un cas d'insuccès de la vaccination antityphique, Jour. de méd. de Bordeaux, 1913, xxiii, 371.

THE TYPHOIDIN REACTION

One of the greatest difficulties that has been present in determining the protective value of typhoid immunization as a whole has been the impossibility of determining the protection of a given group of persons by other means than the careful study of morbidity statistics among vaccinated people over a long period of years (Firth[66]). This difficulty delayed the final acceptance of typhoid immunization for at least eight years (1896-1904). Still less have we any means of determining whether or not a given person who has been vaccinated is actually protected against typhoid fever. We have already described in full the skin-test which Gay and Force[67] have employed as of presumptive value in testing resistance to typhoid infection.

A new summary of our results with the typhoidin may be of interest: In forty-four cases giving a definite history of typhoid fever from four and one-half months to forty-one years previously, forty have given a clear-cut skin-test to the concentrated growth of the bacillus. Of the four negative tests, two gave a strong skin-reaction to a similar preparation made from *B. paratyphosus A.* which we have found not to occur in anything like the same intensity in those cases that react to typhoidin. Only one of the remaining negative cases was further tested with the paratyphoidin solution and therefore only one of two cases was unexplainably negative and 95 per cent. (forty of forty-two) positive. We have a growing set of observations on comparative tests with two paratyphoidin solutions (A and B) in recovered typhoid and parathyphoid cases, and in those vaccinated against typhoid, which we may wish to present at a later time; they apparently show certain interesting group reactions.

Of the controls giving no history of typhoid fever, thirty-eight out of forty-four, or 86 per cent., have been clearly negative. Only five of these control cases (11 per cent.) reacted distinctly positively, and we believe may be explained as cases of aborted or mild, undiagnosed typhoid fever. This explanation is rendered probable by the observation, brought to our attention by Dr. Edward von Adelung, of a perfectly controlled clinical experiment on this subject. The observer, Dr. von Adelung, was a member of a family party of four which visited Germany nineteen years ago. About two weeks after drinking at a suspected water source, two of the members came down with a fever which ran the typical course of typhoid and was so diagnosed. The other two members of the party at the same time

66. Firth: A Statistical Study of Antienteric Inoculation, Jour. Royal Army Med. Corps, 1911, xvi, 589.

67. Gay, F. P., and Force, J. N.: A Skin Reaction Indicative of Immunity Against Typhoid Fever, Studies in Typhoid Immunization III, THE ARCHIVES INT. MED., 1914, xiii, 471.

had mild symptoms, lasting one and three days, respectively, and consisting of headache, fever, malaise and a sensation of flushing, which they regarded as abortive typhoid. Neither of the latter two persons gave any other history of typhoid fever. When the skin-reaction was applied to the two cases that had run the typical typhoid course and to the two that had passed through the "abortive" attacks, all gave positive results. This well-controlled, natural experiment would seem to prove that the supposedly normal cases that react to typhoidin may well be those that have had abortive or undiagnosed typhoid fever in the past.

Our experience with the skin-reaction in those who have been vaccinated with various typhoid vaccines, including our own, has shown us that the majority of those who have been treated within a year and a half or two years may be expected to give a positive reaction, provided always that the last injection has been given at least a month previously. After two years the reaction is less likely to be positive. This experience with the skin-test would correspond very closely with what has been found clinically to be the usual duration of artificial typhoid immunity. We have felt justified, then, in recommending to students who have taken the typhoid vaccine previously that they should have a skin-test applied subsequently at intervals and that if it turned out to be negative they should repeat the treatment.

Since our last communication on the typhoidin test, we have been led to modify the preparations of the solutions employed. We found that some of the original preparation of typhoidin after four or five months failed to produce a positive reaction in cases in which we had been led to expect it. That this failure was due to a deterioration in the product was evidenced by subsequent positive results obtained in six such cases by means of a new preparation. Although such deterioration does not occur for a considerable time with tuberculin, it is known to take place in mallein and abortin. In the latter two cases and in tuberculin as well, better reactions have been obtained with preparations purified by alcohol precipitation and dried (compare Haring[68] and Meyer and Hardenbergh[69]). A similar preparation has been made by precipitating the original typhoidin with twenty volumes of alcohol, filtering, washing with absolute alcohol and ether, and then drying on porcelain plates over a vacuum with sulphuric acid. The control solution, 5 per cent. glycerin-bouillon evaporated to one-tenth volume, was treated in a similar manner. Ten c.c. of the

68. Haring, C. M., and Bell, R. M.: The Intradermal Test for Tuberculosis in Cattle and Hogs, Univ. Cal. Pub. Agric., 1914, Bull. 243, p. 94.

69. Meyer and Hardenbergh: On the Value of the "Abortin" as a Diagnostic Agent for Infectious Abortion in Cattle, Jour. Infect. Dis., 1913, xiii, 351.

original typhoidin yielded 0.78 gm. of dried powder when the culture had been grown for five days before evaporating. The dried powder from the same amount of a control solution gave only 0.5 gm. There is every reason to believe that this dried typhoidin will keep its potency undiminished for at least a considerable time. Our observations, however, extend only to its trial over a period of two months. We dissolve a small amount in carbolated saline equivalent to the original volume of concentrated typhoidin or even to a double concentrated solution and find that when kept in a cool place, it gives very good reaction in typhoid immunes for at least a month.

Our suggestion and growing belief that this skin reaction with typhoidin is a real measure of the protection that the person enjoys against typhoid fever is strengthened by observations on our immunized rabbits. We have already shown that the agglutinin titer is no indication of the resistance of a given animal to infection, and observations on the Widal reaction in man tend to the same belief (Ruediger and Hulbert[71]). The typhoidin reaction, on the other hand, is positive in that category of persons who are known to be protected against typhoid fever, namely, typhoid recoveries; it does not occur in persons who give no history of the disease, except in a small percentage that may reasonably be suspected of having had an abortive attack. The reaction further occurs in the majority of those persons who have been vaccinated against typhoid within the last two years and then gradually disappears. We had scarcely hoped to show differences in typhoidin reaction between incompletely and perfectly immunized rabbits as tested by our method of infection, which is, after all, a violent one, but our results in this respect have exceeded our anticipations. It is, however, easy to demonstrate fundamental differences between normal and immunized rabbits by the intradermal reactions. Cutaneous reactions were first tried, but abandoned as unsatisfactory.

With the intradermal reaction, performed by producing a tiny bleb under the skin by a short needle carrying typhoidin, a distinctive reaction is produced in rabbits that have been artificially immunized, but not in controls. In each case a patch on the abdominal surface is shaved the day before the test and two blebs produced under the epidermis by injecting in the one concentrated glycerin bouillon and in the other typhoidin. In the normal animal there is little or no difference between control and typhoidin spot. Both are usually surrounded by a red zone of a few millimeters, and there may be slight induration. In the animal that has previously been treated by typhoid

71. Ruediger and Hulbert: Is Dried Blood as Reliable as Fresh Serum in Making the Widal Test? Am. Jour. Pub. Health, 1914, iv, 113.

vaccines there is a sharp difference between the two spots. The typhoidin spot, as early as five hours, but with increasing regularity and intensity to twenty-four hours, becomes surrounded by a red areola, is indurated, and quickly forms a firm, hard nodule with a yellowish, slightly softened center, from 2 to 5 mm. in diameter. This nodule persists at least for several days and may last two or three weeks. It is characterized histologically by an infiltration of lymphoid cells with which are mixed a few polymorphonuclear leukocytes.

Five normal rabbits tested in this manner gave negative reactions. Of thirty-five more or less perfectly immunized rabbits, twenty-six (74 per cent.) reacted positively. The differences in reaction between animals effectively immunized against our method of producing carriers and those which are not so fully protected is a relative matter when viewed in the aggregate, but in individual experiments is more striking. Thus, dividing our treated animals in respect to a negative or positive intradermal test and then comparing the results with their carrier condition or recovery on infecting the following day, we find that of nine with a negative intradermal test, seven, or 78 per cent., became carriers; of twenty-four with a positive intradermal test, thirteen, or 54 per cent., became carriers, a distinct, though only relative indication of agreement between the test and the absolute protection.

In Table 4 is given the comparative immunizing value of four different vaccine preparations. Before the test inoculation, the intradermal test was applied to these animals with the following results as compared with the eventual carrier conditions:

Group 1, vaccinated with whole unsensitized vaccine (three animals): intradermal test, 3 negative; carriers produced, 2; recovered, 1.

Group 2, vaccinated with unsensitized sediment (five animals): intradermal test, 4 positive; carriers produced, 3; recovered, 2.

Group 3, vaccinated with whole sensitized vaccine (three animals): intradermal test, 3 positive; acute death (anaphylaxis), 1; recovered, 2.

Group 4, vaccinated with sensitized sediment (four animals): intradermal test, 4 positive; carriers produced, 1; recovered, 3.

These results show a distinct relation between a positive intradermal test and recovery and between a negative intradermal test and establishment of the carrier state.

MECHANISM OF THE TYPHOIDIN REACTION

We do not intend, at this time, to discuss fully the experimental evidence and the hypotheses that have been brought forth to explain the generalized and localized reactions that follow the injection or application of bacterial extractives like typhoidin in infected or immunized individuals. Of these reactions, the one to tuberculin has natur-

ally been most studied. Some of the most interesting and debatable questions that seem to have arisen in connection with the tuberculin reaction are, first, whether or not it is in reality a reaction exactly similar to the anaphylactic reaction with serum, and second, whether it is due to an interaction of antigen and antibody accompanied, it may be, by alexin fixation (Wassermann and Bruck). As regards the local, cutaneous test we have the further possibility that the antibodies, hypothetically furnished to unite with the antigen, may be furnished from the general circulation or due to local cellular response (von Pirquet). There is certainly evidence in the case of typhoid recoveries that the protection is cellular rather than humoral, and the relative inefficiency and short duration of artificial vaccination against typhoid fever may well be due to the fact that the immunity is not sufficiently cellular in type.

It seemed possible to attack certain of these problems in relation to the localized typhoidin test. The first question that arose was whether or not the reaction was due to the local combination of antigen and antibody. As has already been shown, the sensitized vaccine which we have employed gives less reaction, both general and local, than the untreated vaccine when used in the prophylactic treatment of human beings. We should not, therefore, expect that the local application of a sensitized vaccine in normal individuals would call forth any, or at any rate, not so marked a response as the untreated vaccine. And yet, it was argued, if the local typhoidin reaction in an immunized individual is really due to a combination of antigen and antibody, sensitized vaccine, which is just such a combination, might produce a local reaction in a normal individual.

EXPERIMENT 6.—Suspensions of unsensitized, dried Typhoid Vaccine 4 and of sensitized, dried Typhoid Vaccine B were made in the proportions of 5 mg. of vaccine to 1 c.c. of carbolated saline solution.

On normal rabbit 319 were produced intradermally small blebs of the foregoing sensitized and unsensitized suspensions. Twenty hours later the following condition was observed:

"Vaccine 20 bleb (unsensitized) measures 3 by 5 mm., is not markedly raised, and has no yellowish center.

"Vaccine B bleb (sensitized) measures 6 by 5 mm., is surrounded by an areola that is indurated, markedly higher than the other point, and with large, firm, raised yellowish papule, which resembles in all respects the positive skin-reaction obtained in immunized animals except that the surrounding areola is not quite so extensive."

Normal Rabbit 322, treated in the same manner, showed slightly more reaction about the unsensitized spot than the sensitized, but no distinct papule was present as in Rabbit 319.

A skin test with the two preparations was then tried on two normal, unvaccinated human beings. No reaction was noted with either spot. Since the intradermal test is recognized as being more sensitive, it was next tried on normal persons.

Thus, when comparative intradermal tests with sensitized and unsensitized vaccine were made on two normal rabbits, one of the two showed a much more marked reaction to the sensitized vaccine than to the unsensitized, the sensitized vaccine reaction resembling in all respects that produced in an immunized animal by the injection of typhoidin.

Of four human beings that were similarly tested by intradermal blebs, three showed a very characteristically increased reaction to the sensitized vaccine over the unsensitized vaccine. In only one case were the reactions produced by the two suspensions similar, and both were so marked as to suggest the possibility of an overlooked typhoid history. We regard this experiment, then, as distinctly indicating that the local reaction to typhoidin is due to an interaction of antigen and antibody.

TABLE 7.—NORMAL PERSONS TESTED BY INTRADERMAL TEST WITH SENSITIZED AND UNSENSITIZED TYPHOID VACCINES*

No.	Reaction to Sensitized Vaccine (5 mg. to 1 c.c.)	Reaction to Unsensitized Vaccine (5 mg. to 1 c.c.)
1	24°. Large indurated area 80 by 100 mm.	Same as other bleb
2	18°. Red area 50 by 58 mm. (Foregoing solutions diluted one-tenth concentration) (0.5 mg. to 1 c.c.)	2.2 by 2.2 mm. Less red
3	24°. Demarcated, red, slightly indurated area, painful 7.1 by 5.4 mm.	Indefinite, slightly red area, 4.2 by 2.1
4	24°. Red, demarcated area 6.2 by 5.2 mm.	Less red, not demarcated, 4.9 mm.

* The tests were applied by making blebs, one on each upper arm.

One further point on the mechanism of the typhoidin skin-test has been brought out by experiments on rabbits. Having reached a logical assumption that the skin-test is due to the interaction of antigen and antibody in the immune individual, it remained to show whether the antibody furnished for this combination comes from the circulating blood or is due to local tissue reaction. If the first hypothesis is true, immune serum should transfer the reactionability to a normal animal and an immune animal that reacts should at least partially lose this power if exsanguinated and transfused with normal rabbit blood. Both these conditions were found possible of realization.

EXPERIMENT 7.—The results of this experiment may best be described in parallel columns (Table 7).

This experiment, as well as another one carried out under the same conditions and with the same results, shows clearly that the abstraction of blood from an immune animal that reacts strongly to an intradermal test of typhoidin renders it almost completely insusceptible, whereas a control immune reacts a second time on corresponding days. The blood of the immune animal transfers the susceptibility to the typhoidin reaction to a normal animal, which has previously reacted negatively. We may conclude, therefore, that in the case of immunized rabbits, and perhaps also in the case of human beings artificially immunized against typhoid fever, the skin-reaction may be accounted for by the interaction of the antigen with antibodies in the circulating blood. We do not wish at all to suggest that the skin-reaction in typhoid recoveries is due to the same mechanism. The absence of

TABLE 8.—TRANSFER OF REACTIBILITY TO SKIN REACTION BY TYPHOIDIN

Date	Typhoid Immune Rabbit 298	Typhoid Immune Rabbit 293	Normal Rabbit 329
April 7...	Intradermal test applied	Intradermal test applied	Induration test applied
April 8...	Reaction strongly positive	Reaction strongly positive	Reaction negative
April 9...	.....	Bled for 50 c.c. and rapidly transfused from a normal rabbit	
April 10...	Intradermal test applied	Intradermal test applied	Given 20 c.c. serum from Rabbit 293
April 11...	Reaction strongly positive	Reaction negative; slight induration; no papule	Intradermal test applied
April 12...	.....	.....	Reaction distinctly positive and increasingly so to seventy-two hours; induration; papule and yellow center.

demonstrable antibodies in the majority of these cases, as well as their superior type of immunity, would lead one to think that the skin reaction may be cellular rather than humoral in character.

SUMMARY AND CONCLUSIONS

The history of the development of artificial immunization against the typhoid bacillus in animals and human beings is reviewed, and some of the many preparations of the typhoid bacillus used for this purpose are discussed. Particular attention is drawn to the reputed advantages of living sensitized typhoid vaccine (Besredka) as opposed to other types of vaccine. The many vaccines that are still being

advocated indicate that the best vaccine has not yet been found and that the best method of proving which is the best vaccine has not been determined.

In the present article we explain again the method of testing typhoid immunity by means of an artificial typhoid-carrier state in the rabbit, and again advocate it as the best test for this purpose short of experimentation on anthropoid apes. We find this method admirably suited to indicate slight differences in typhoid preparations as regards their ability to produce an immunity against a subsequent typhoid carrier condition produced by intravenous injection of living typhoid bacilli. In successive series of experiments we have been able to show by this method, first, that untreated bacteria, killed and precipitated by alcohol, dried, ground and weighed, do not protect so well as typhoid bacilli that have been sensitized by an immune serum and subsequently treated in the same manner. Second, it has been possible to show in the case of the unsensitized, dried bacteria that the sediment of bacterial bodies freed from the supernatant, endotoxic fluid as prepared from these dried cultures contains the immunizing principle almost in its entirety. The sediment of either sensitized or unsensitized cultures protects not only better than the supernatant fluid from these sediments, but actually better than the whole, unseparated mixture. Third, we find that alcohol-killed, sensitized cultures protect almost as well as living, sensitized cultures and that the sediment of alcohol-killed, sensitized cultures protects better than living, sensitized cultures.

Sensitized cultures of the typhoid bacillus, whether whole or as sediment, produce little or no reaction in human beings with the possible exception of those who have previously suffered from typhoid fever or have been immunized. By comparing our results with those of certain other observers, we conclude that a considerable degree of reaction, both local and general, is avoided by the use of these sensitized cultures, which possess the further advantage, so far as our experimental work can determine, of producing a more durable type of immunity.

We recommend vaccination at short intervals (two days) in human beings, which is rendered quite possible with the mild vaccine we employ, and is evidenced from animal experimentation, as giving rise to less toxic effect and to fully as durable an immunity as vaccination at longer intervals. This type of vaccination has the further advantage of completing the prophylactic treatment of three injections within a week.

We further believe that a polyvalent vaccine derived from strains of the typhoid bacillus isolated in the vicinity of cases that are to be

treated is advantageous, judging largely from the work of other observers. A further advantage in the use of sensitized cultures is that a polyvalent vaccine, no matter how recently the strains may have been isolated, is also almost entirely free from untoward effect. We have no direct experimental evidence bearing on the superior immunizing value of such a polyvalent vaccine. The dosage of vaccines in human beings which we recommend is empirical and based on the usage of other experimenters.

We recommend, then, for prophylactic immunization against typhoid fever, three injections of the sediment of a dried, ground, sensitized culture of several local strains of the typhoid bacillus mixed together, given at two-day intervals and in a dosage of $\frac{3}{32}$ mg. of the original dried culture, which corresponds, as has been determined, to a dosage of approximately 750 million living typhoid bacilli.

Our own experimental and clinical results, as well as the work of many other investigators, leads us to regard the agglutination reaction with the serum of immunized animals and human beings as by no means indicative of the degree of protection afforded against infection with the typhoid bacillus. Of far more prognostic significance is the skin test with the typhoidin solution, which Gay and Force have recently described. Further summaries of the results with this test show that this reaction is positive in the majority of recovered cases of typhoid (95 per cent.), even as far back as forty-one years, and that it occurs in only about 11 per cent. of persons who give no history of typhoid or of typhoid immunization. Distinct evidence is brought forth that indicates that these supposed normals reacting to typhoidin have suffered from an abortive or undiagnosed typhoid fever. Persons immunized with various types of typhoid vaccine react in the majority of cases for about two years and then become more frequently negative. We regard a negative skin-test after vaccination as indication for revaccination.

That a positive typhoidin test bears a distinct relation to protection against typhoid fever is further indicated by similar reactions produced intradermally in our immunized rabbits. In spite of our more violent method of artificial infection as against natural infection in typhoid fever, we find that animals that resist becoming carriers show a higher percentage of positive intradermal tests to typhoidin administered the day before; whereas those that are not immunized sufficiently to resist the carrier state more frequently show a negative typhoidin reaction.

We suggest the use of dried, alcohol precipitate from typhoidin solution as a stock from which the test solution may be prepared, since it has been found that the original typhoidin solution deteriorates within a few months.

Experiments dealing with the mechanism of the local typhoidin reaction indicate that it is due to an interaction of antigen and antibody, as is shown by the fact that the *intradermal* test of a sensitized vaccine will produce a characteristic reaction in human beings, whereas the corresponding amount of an unsensitized vaccine produced no such reaction. The paradox of producing a more severe local reaction with the sensitized vaccine, which has been found to produce less violent reactions when used for immunization, is only apparent. Further experiments with rabbits indicate that in the condition of artificial immunization against the typhoid bacillus, the antibodies which unite with the antigen to produce the local test are in the circulating blood, as is indicated by the passive transfer to a normal rabbit by means of serum from an immune rabbit of susceptibility to this reaction. This is further evidenced by the extraction of blood from the immunized rabbit and a corresponding loss of reaction. We do not regard this experiment, however, as indicating the circulatory nature of the antibodies in the recovered typhoid persons who almost invariably react to typhoidin.

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A CASE OF DIABETES INSIPIDUS*

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Johann Peter Frank[1] in 1794 first gave a definition for diabetes insipidus when he described it as a long-continued abnormally increased secretion of non-saccharine urine which is not caused by a diseased condition of the kidneys. Thomas Willis,[2] however, had previously recognized a distinction between a saccharine and a non-saccharine diabetes by discovering the fact that, in certain cases of polyuria, the urine had a sweet taste. The two first cases of diabetes insipidus, reports of which were collected and published in English, were recorded by Dr. Simmons[3] two years before Frank's work appeared, and are of such historical importance that it is of interest to quote them in some detail.

AN ACCOUNT OF TWO CASES OF POLYDIPSIA, OR EXCESSIVE THIRST

"Almost all the modern nosologists have introduced into their systems a disease, to which, on account of the excessive thirst that forms its characteristic symptom, they gave the name of polydipsia; but in none of the examples of it they have been able to collect does it appear to have been idiopathic; and Dr. Cullen expressly says that it is almost always symptomatic. A very curious instance, however, of such an affection, apparently depending on a peculiarity of temperament, or what is called idiosyncracy, occurs in a woman now living in Paris, of whose case the first account was given by M. Bessejon de la Chassagne, in a letter which we shall here translate from the Paris *Journal* of May 1, 1789."

"PARIS, April 18, 1789.

"To the Authors of the Journal,

"Gentlemen:—You will interest equally the humanity and curiosity of the public, by inserting in your Journal the following anecdote, which, without doubt, will be considered as a phenomenon. I have assured myself, with the most scrupulous accuracy, of the facts which I announce, but I leave to the reader to explain their nature and cause.

"Catherine Bonsergent has been remarked from her tenderest years. A burning thirst, a drought without example, with which she has been con-

* Submitted for publication May 19, 1914.
* From the Medical Clinic of the Peter Bent Brigham Hospital, Boston.
* Reported at a meeting of the Boston Society of Medical Sciences, April 14, 1914.

1. Frank, Johann Peter: De Curandis Hominum Morbis, iii, Class V (Profluvia), Ordo I (prfl. serosa), Genus II (diabetes), p. 28, Florence, Coen et Socios, 1832.

2. Willis, Thomas: De Diuresinimia, ejusque remedio, et speciatim de Diabetes, in cujus Theoriam et Therapiam inquiritur, Opera Omnia, Pharmaceutice Rationalis, Sectio IV, Caput III, Henricum Wetstenium, Amsterdam, 1682.

3. Simmons: Medical Facts and Observations, London, J. Johnson, 1792, ii, 73.

tinually afflicted from the time of her birth, have always fixed on her the attention of persons of observation. Her parents, after having entrusted the first care of her to a nurse, took her home when she was three years old. It was not long before they observed that an extraordinary quantity of water was consumed in the house, and at length discovered that their daughter drank, every day, to the amount of two pailfuls. In summer she drank the first water she could meet with, in winter she had recourse to snow and ice, and she was always careful to reserve as much as would enable her to drink abundantly during the night.

"At the age of twenty-two years she married one Fery, a cobbler, from whom she contrived to conceal her complaint till after their marriage. She has had by him eight children, three of whom are still alive, and she is now pregnant with a ninth. What appears particularly extraordinary is, that during her lyings-in instead of having recourse to such food and liquors as would seem most likely to strengthen her, she chooses rather, for the sake of satisfying her thirst, which at those times is more intense, to drink almost without interruption, three or four quarts of coldest water. During the severe cold of last winter this woman, who was then pregnant, drank to the amount of four pailfuls of water in twenty-four hours; and her husband being unable to afford the expense of such a consumption, was under the necessity of supplying her with melted snow and ice. The price of a load (two pailfuls) of water, at that time, was six sols; and the quantity she required would have cost him more than he earned by his work.

"This woman has never made use of any sort of strong liquors, and if she drinks only a single glass of wine she feels an uneasy sensation in all her limbs, and seems to be in danger of fainting. She is not dropsical; she even enjoys a pretty good state of health; she voids naturally all the water she drinks, but her urine is extraordinarily foetid. She lives at the Hôtel des Arts, Fauxbourg Saint Martin.

"Signed Bessejon de La Chassagne.
"Prêtre de St. Laurent."

"While the preceding account was preparing for the press the following paragraph appeared in the Lincoln *Mercury* of Friday, December 9, 1791.

"'However extraordinary the following circumstances may appear, it may be depended on as fact: A man who lives with Mr. John Julyan, of Woodstone, near Peterborough, is afflicted with such an immoderate degree of thirst as obliges him to drink the astonishing quantity of three gallons of water a night, and one gallon a day; and what makes this appear still more extraordinary, he has continued this practice for twenty-three years.'

"The facts described in this paragraph bore too striking a resemblance to those he had just before received from Paris not to excite in the editor a wish to see the case more fully and satisfactorily investigated. This has since been done through the kind offices of Sir John Banks, Bart., who being acquainted with a gentleman in the neighborhood of the patient, on whose accuracy he knew he could depend, had the goodness to trasmit to him some queries from the editor relative to this subject, with a request that he would engage in the inquiry.

"In consequence of this request, Mr. Maxwell, the gentleman alluded to, sent for the man to his house, where he remained a whole night, and was carefully attended to. The result of this investigation, which sufficiently established the truth of the facts, we shall here give in Mr. Maxwell's own words.

"Extract of a letter from Mr. George Maxwell to Dr. Simmons, dated Fletton Lodge, near Peterborough, Dec. 18, 1791.

"With respect to the water drinker, who is the subject of your inquiry, and who lives at Stanground, near Woodstone, though he works at the latter place, it happens that Mr. Beal, the person who now looks after my farm, employed him as a thrasher more than twenty years ago. His account of this

man is, that he always drank the quantity he is now said to do, or at least was at that time reputed to drink it.

"As he resided three or four miles from Mr. Beal's habitation, the latter used to make up a bed for him in his house, and Mr. Beal observed that at night he always took a bucketful of water upstairs with him.

"I have a laborer likewise who has worked with him, and who says in mowing time this man always takes four quarts of water out with him from a pump in the village, besides two quarts of beer.

"The man adds, that he has consulted several medical gentlemen about his complaint, but has not been able to get anything that could, in the least, relieve him."

Diabetes insipidus, notwithstanding the long time it has been recognized as a disease, is uncommon. Eichorst[4] saw only 17 cases out of 35,942 patients in the medical clinic at Zürich between 1876-1877. Gerhardt[5] found 55 cases among 113,600 treated at the Charité, Berlin, from 1877 to 1896; and Futcher[6] 7 cases out of 403,535 patients treated in the Johns Hopkins Hospital and Dispensary from 1889 to 1904. Thus from a total of 553,077 cases, there are only 79 of diabetes insipidus, or 14 cases per hundred thousand.

Out of 133 cases of the disease selected from his own observations and the literature up to 1892, Stoermer[7] found that diabetes insipidus affected men more often than women, and that it was commoner in the period of life before forty than later. In children also, Külz[8] showed that boys were more commonly affected than girls. It has a tendency to affect certain families, as stated by Clay,[9] Gee,[10] Lancereaux,[11] and most notably by Weil,[12] who reports seventeen cases in one family during three generations; it is chronic, but is usually not fatal on its own account.

The cases which have come to necropsy have shown various pathological conditions. The most common anatomical lesion has been brain tumor of either a malignant or of an inflammatory nature. The symptoms occur most frequently in association with tumors which involve the floor of the fourth ventricle or are in its immediate vicinity, or which are in the hypophyseal gland. On the other hand, as shown by Simmonds,[13] tumors occur in this situation without establishing the symptom-complex.

In the literature, repeated reference is made to the importance of either congenital or acquired syphilis as an etiological factor. In such

4. Eichorst: Cor.-Bl. f. schweiz. Aerzte, 1888, xviii, 393.
5. Gerhardt: Nothnagel's Specielle Pathologie und Therapie, 1900, vii, 7.
6. Futcher: Tr. Assn. Am. Phys., 1904, xix, 247.
7. Stoermer: Inaug. Diss., Kiel, 1892.
8. Külz: Handbuch der Kinderkrankheiten, 1878, iii, Ersste Hälfte, p. 285.
9. Clay: Lancet, London, 1889, lxvii, part 1, p. 1188.
10. Gee: Saint Bartholomew's Hosp. Rep., 1877, xiii, 79.
11. Lancereaux: Thèse de Paris, 1869.
12. Weil: Virchow's Arch. f. path. Anat., 1884, cxv, 70.
13. Simmonds: München. med. Wchnschr., 1914, lxi, 27.

cases the underlying process is usually either a gumma in the floor of the fourth ventricle or in the hypophysis, a basilar meningitis, or an endarteritis with connective tissue proliferation. A certain number of cases have been reported in which no demonstrable anatomical changes existed.

These facts have led to the classification of diabetes insipidus into two types:

1. Symptomatic, including the cases in which there is evidence of organic disease either in the brain or elsewhere, the lesion being considered the cause of the diabetic symptoms.

2. Primary or idiopathic cases, including those in which there is no evident organic basis for the disease.

The part that the kidney plays in causing the characteristic symptoms of diabetes insipidus has been studied from various points of view. Claude Bernard,[14] Eckhard,[15] and more recently Kahler,[16] have attacked the problem of the physiology of polyuria by animal experiments. Their results in brief show that wounds of the floor of the fourth ventricle frequently produced polyuria, but that in rabbits, at least, there was no definite point which would produce a pure "hydruria." Section of the spinal cord at the level of the sixth or seventh cervical vertebra, as well as at higher levels, produced an immediate and persistent suppression of urine. Sections below those levels gave less certain and less prompt results, whereas sections of the cord below the twelfth dorsal vertebra had practically no effect whatever on the secretion of the urine. Schäfer and Herring[17] have studied the action of the secretion of the hypophysis. They found that a systemic rise in the blood-pressure was produced by intravenous injections of the gland extract, with a specific dilating effect on the renal blood-vessels. They believed that pituitary extract had a pharmacological action on the kidney analogous to that of caffein.

It may be concluded, therefore, that the nerve fibers which supply the kidneys pass from the medulla to the level of the sixth or seventh cervical vertebra, where they leave the cord in the uppermost thoracic nerves and pass along the wall of the aorta and renal arteries to the kidneys; and that an experimentally produced polyuria is an irritation phenomenon or is due to an increased amount of the secretion of the pituitary gland in the circulating blood.

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14. Bernard: Leçons sur les propriétés physiologiques et les altérations pathologiques des liquides de l'organisme, Paris, 1858, I, 397.

15. Eckhard: Beiträge zur Anatomie u. Physiologie, 1869, iv, I, 153; 1870, v, 147; 1872, vi, 51.

16. Kahler: Ztschr. f. Heilk., 1886, vii, 105.

17. Schäfer and Herring: Phil. Tr. Roy. Soc., London, 1906, Series B, cxcix, 1.

Clinical studies on the action of the kidney in diabetes insipidus have been made by many observers with varying interpretations. Erich Meyer,[18] Tallqvist,[19] Socin[20] and others believed that the disease was caused by a primary inability of the kidney to excrete urine of normal concentration. Therefore the urine was dilute, of low specific gravity, and of low molecular concentration as estimated by the freezing-point. When sodium chlorid or nitrogen was added to the diet, these substances were excreted by a marked polyuria, or were compensated for by the retention of other substances. Theocin did not act as a diuretic, but stimulated the concentrative powers of the kidney for a short time, so that during its administration an increased amount of chlorid was excreted, which was later retained after the drug was omitted, until the chlorid balance was regained. Finkelnburg,[21] by making observations at frequent intervals felt that the concentrative power of the kidney was not lost, agreeing with Schwenkenbecher[22] and Forschbach and Weber,[23] who considered diabetes insipidus a disease in which the kidney under pathological conditions of stimulation excreted abnormally large amounts of urine. Frank[24] observed a case due to a bullet which lodged in the pituitary fossa. From a review of the literature and this observation, Frank thought that many cases of essential diabetes insipidus could be explained by hyperfunction of the pituitary gland with a resultant diuretic action. To meet the argument that an increased blood-pressure and other pharmacological activities of this substance were not observed, he replied that smooth muscle had an antagonistic action against pituitary extract, while its diuretic effect was unhindered. Cushing's[25] observations emphasized the importance which this gland plays in producing the disease. Ebstein,[26] finally, considered that the condition was due to a primary polydypsia with resultant polyuria because if the kidneys were primarily at fault, when the fluid intake was diminished, the specific gravity would not increase, whereas in many of the observations recorded, the opposite has taken place and the concentration and specific gravity has risen as in normal individuals.

18. Meyer: Deutsch. Arch. f. Klin. Med., lxxxiii, 1; Deutsch. Klin., 1910, xiii, Ergnzgsbd., ii, 271; Ztschr. f. klin. Med., 1912, lxxiv, 352.
19. Tallqvist: Ztschr. f. klin. Med., 1903, xlix, 181.
20. Socin: Ztschr. f. klin. Med., 1913, lxxviii, 294.
21. Finkelnburg: Deutsch. Arch. f. klin. Med., 1910, c, 33.
22. Schwenkenbecher: München. med. Wchnschr., 1909, lvi, 2564.
23. Forschbach and Weber: Ztschr. f. klin. Med., 1911, lxxiii, 221; Forschbach: Ztschr. f. klin. Med., 1913, lxxvii, 153.
24. Frank: Berl. klin. Wchnschr., 1912, xlix, 393.
25. Cushing: Med. Communications, Mass. Med. Soc., 1913, xxiv.
26. Ebstein: Deutsch. Arch. f. klin. Med., 1909, xcv, 1.

On the whole, from the literature it appears that diabetes insipidus is usually of the symptomatic type, though it may be idiopathic. The exact cause of the polydipsia and polyuria is undetermined; certain observers believe that the kidney's concentrative powers are at fault, others that the polyuria results from abnormal thirst, and others that the kidney is under a pathological condition of stimulation which may be due to an increased internal secretion of the hypophysis with resultant polyuria and polydipsia.

REPORT OF CASE

The following case is reported on account of the rarity of the condition and because it seems possible that studies in renal function according to present methods may be of interest in this disease.

Alfred L. (Peter Bent Brigham Hospital Medical No. 911) of Syrian descent, 12 years old, white, a schoolboy, born in Jamaica, W. I., entered the hospital with the following history:

His father is living and well; he has four brothers and one sister who are living and well. His mother is dead, but the patient is unable to state from what cause. To his knowledge, there is no family history of polyuria or polydipsia.

He has lived in Boston as long as he can remember. In 1909 (five years ago) he had measles, scarlet fever and chicken-pox. Otherwise he has always been well.

He is in the fifth grade in school. His appetite is not abnormal, his digestion is good, his bowels are constipated so that they move only twice a week. His eyesight is good. He has had no headache. He drinks two or three cups of coffee daily, but no tea.

Five months ago he first noticed that he had to get up at night to urinate. Since then he has complained of an increasing thirst and frequency of micturition, so that at present he urinates about ten times a day, and four or five times at night, passing more than a pint each time. He has not lost weight or strength, and has no pain with micturition.

He is a fairly well-nourished boy, of normal skeletal and sexual development. There are small glands palpable in the neck, and in the epitrochlear and inguinal regions. The ears, nose, mouth and throat are not remarkable. The heart is not enlarged to percussion; no murmurs are heard, and the aortic second sound is normal. The systolic blood-pressure is 106; the diastolic is 70. The lungs are resonant throughout; breath and voice sounds are not remarkable; no râles are heard. The abdomen is negative.

Neurological examination reveals nothing abnormal. The fundi oculorum are negative, no contraction in the form field of either eye is demonstrable, but determination of the color fields is unsatisfactory because of the patient's lack of cooperation.

An x-ray of both kidneys, both ureters and the bladder shows no abnormal shadows. An x-ray of the skull shows rather marked cerebral impressions, but is otherwise negative. The sella tursica shows normal size and shape. Its largest diameter is about 10 mm., and it is about 7 mm. deep. The anterior and posterior clinoid processes are distinct. The development of the skeleton of both hands corresponds to the age.

Examination of the patient's blood shows a white count of 12,000, hemoglobin of 75 per cent. Smear shows slight achromia and anisocytosis of the red corpuscles. A differential count of the white cells gives 61 per cent. of neutrophils; 31 per cent. of small mononuclear cells and 3 per cent. of large

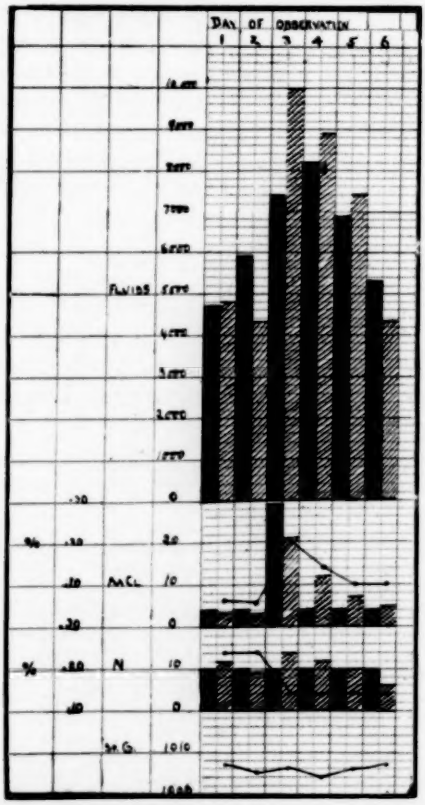


Chart 1.—Tests for renal function. One c.c. of phenolsulphonephthalein solution was injected intramuscularly, and the excretion at the end of one hour and two hours was determined colorimetrically (30 per cent. for one hour, 60 per cent. for two hours) according to Rowntree and Geraghty (THE ARCHIVES INT. MED., 1912, ix, 284). Potassium iodid, 0.5 gm., was given by mouth, and the length of its excretion time (twenty-four hours) found by Sandow's test. Lactose, 2 gm. in 20 c.c. of distilled water, was injected intravenously and its elimination followed both qualitatively by Nylander's test, and quantitatively by polarimetry (four-hour excretion, 60 per cent. recovered). The diastase content of the urine was estimated (1 c.c. urine digests 7 c.c. of 0.1 per cent. starch solution in half an hour) by Wohlgemuth's method (Wohlgemuth and Noguchi: *Berl. klin. Wchnschr.*, 1912, xxiii, 1069). The blood nitrogen was estimated (17.5 mg. per 100 c.c. of blood) by Folin and Denis' micromethod (*Jour. Biol. Chem.*, 1912, xi, 527). The chlorid determinations were made by Volhard's method, and the nitrogen determinations by Kjeldahl's method. In this and the following charts, unless otherwise stated, the solid black column represents intake of fluid, sodium chlorid or nitrogen, and the cross-hatched column output. The dotted line represents concentration of chlorid or nitrogen per hundred c.c. of urine, and specific gravity.

mononuclear cells. The patient's stool is well formed, normal, contains no occult or free blood, and no ova.

The Wassermann reaction of the patient's blood-serum is strongly positive. The spinal fluid, however, is under normal pressure, contains 2 cells per cubic millimeter, and reduces Fehling's solution. The globulin and Wassermann reactions are negative.

While under observation the patient has passed from 4 to 10 liters of urine a day with a correspondingly large fluid intake. Repeated examinations of the urine have shown a low specific gravity, no albumin or sugar, and no blood, pus or casts in the centrifuged sediment.

On the whole the history is characteristic of diabetes insipidus. The only positive clinical finding is a Wassermann reaction in the blood-serum. Since the cerebrospinal fluid is normal, there is no evidence of any meningitis. Thus the case clinically must be considered as an idiopathic diabetes insipidus, or, if symptomatic, due to a small lesion in the brain, possibly of a syphilitic nature, without any focal manifestations.

Various observations have been made on the patient's renal functions which are discussed with illustrative charts. During the entire period, unless otherwise stated, the patient has been given daily as much water as he chose, and has eaten a constant diet containing 10 gm. of nitrogen and 4 gm. of sodium chlorid in such form as to yield a total of 2,000 calories.

Schlayer[27] and his co-workers have assumed that different portions of the renal structure have selective excretory activities which can be tested individually. Experiments have shown that water and lactose, when injected intravenously, are put out mainly through the action of the glomerular vessels, while sodium chlorid and potassium iodid are concentrated in the tubules. Normally large amounts of salt are put out by one of two methods. If the sodium chlorid is given without extra water it is excreted by increased concentration in the urine; if given with additional water it is excreted partially by increased concentration and partially by an increased amount of urine. In severe tubular injury with a normal vascular system, salt is retained in the body, since the tubules are impermeable, and potassium iodid is excreted over a long period of time. When the glomerular function is abnormal, the excretion of water is interfered with. There may be polyuria due to a hypersensitive condition of the blood-vessels by which they respond continuously to the small amount of diuretic substances contained in food, and exaggerated by the addition of sodium chlorid; or there may be oliguria due to a torpid condition of these vessels as a result of which they are unable to excrete the usual amount of fluid. Urine of low specific gravity, therefore, is of two sorts. A "tubular hyposthenuria" occurs when the renal vessels are not hypersensitive, and the tubules have lost their concentrative powers. "Vascular hyposthenuria" is found when the vessels are hypersensitive and respond with polyuria to the diuretic properties of sodium chlorid. Between these two extreme types are various gradations.

27. Schlayer: Deutsch. Arch. f. klin. Med., 1911, ci, 333; 1911, cii, 311.

In our case, according to these premises, there existed a constant "hyposthenuria." The total renal function was not affected, since the phthalein elimination was normal, and there was no accumulation of nitrogen in the blood. The diastase in the urine was low, but was not entirely lacking, agreeing with the observations of Benczur[28] and Geyelin,[29] and opposed to Strauss[30] and Rosenthal,[31] who found it absent in this condition.

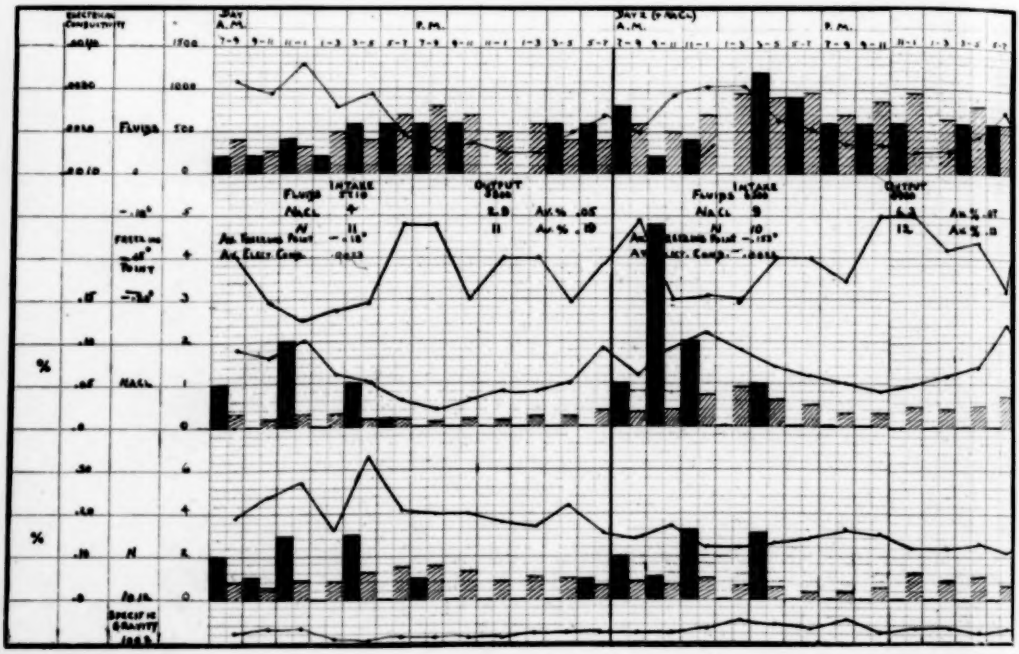


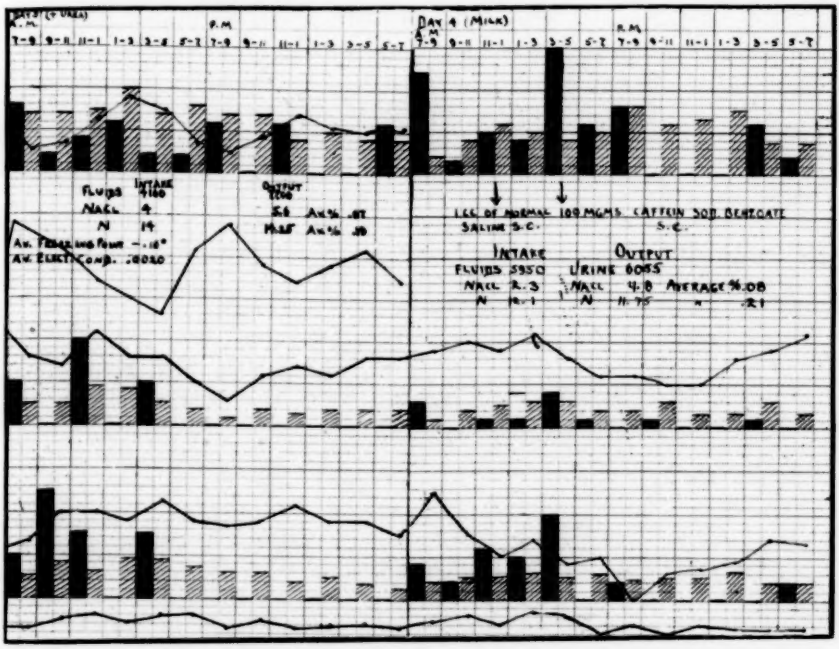
Chart 2.—The effect of sodium chlorid, urea, and other diuretics on two-hourly specimens of urine by Kohlrausch's method. The electrical resistance was measured in reciprocal ohms per centimeter cube Messungen, Englemann, Leipsic, Ed. 3, 1910, p. 473). It gives the author great pleasure to thank Dr.

When 25 gm. of sodium chlorid were given, there was slight increase in its concentration in the urine for the following forty-eight hours, which would suggest that the tubules were still functionally active. This was confirmed by a normal excretion time for potassium iodid. The chlorid, however, had a striking effect on the renal vascular system. For three days following its administration there was a

28. Benczur: Wien. klin. Wchnschr., 1910, xxiv, 890.
29. Geyelin, W. Rawle: A Clinical Study of Amylase in the Urine, THE ARCHIVES INT. MED., 1914, xiii, 96.
30. Strauss: Deutsch. med. Wchnschr., 1912, iv, 163.
31. Rosenthal: Berl. klin. Wchnschr., 1912, xlix, 1265.

polyuria, so that more than 3 liters over the fluid intake was excreted. Lactose came through normally, as might be expected, since Schlayer has already shown that excessive amounts of urine may increase its elimination time. Thus the case showed the typical findings of "vascular hyposthenuria" with hypersensitive vessels.

Since large amounts of sodium chlorid appeared to have such a marked effect on the total amount of urine excreted, it was important



The freezing-point was determined by Beckmann's method, and the electrical conductivity of urine according to Oswald-Luther's tables. (Oswald-Luther: Physiko-Chemische Paul Dudley Lamson for his kindness in making these readings.

to discover the rate of elimination of water and salt; whether smaller amounts of this drug might have the same effect according to Stuber's[32] observations that certain cases of diabetes insipidus reacted to very minute doses, and whether other diuretic substances acted as irritants to the kidney. Accordingly, a four-day experiment was undertaken in which specimens of urine were analyzed at two-hour intervals for nitrogen and sodium chlorid, and in which the freezing-point and electrical conductivity were determined. The first day served as control; on the second day 5 gm. of sodium chlorid was added to the diet; on

32. Stuber: Deutsch. Arch. f. klin. Med., civ, 394.

the third day 10 gm. of urea; on the fourth day the diet was changed to milk, and in the morning 1 c.c. of sterile saline solution was injected subcutaneously, while in the afternoon 100 mg. of caffein sodium benzoate. The results of these observations are shown in Chart 2.

It became evident from the total amount of urine excreted that both sodium chlorid and urea acted in producing a polyuria, that no reaction was obtained from the minute amount of sodium chlorid contained in 1 c.c. of saline solution, and that caffein sodium benzoate possibly had a diuretic action as more fluid was excreted in the hours following its injection than before. From the analysis of specimens from hour to hour it appeared that the rate of fluid excretion under normal conditions was relatively constant and did not bear a definite relation to the amount of fluid ingested. The rate of chlorid and nitrogen excretion, also, was approximately level. When the sodium chlorid and nitrogen were added a diuretic action was obtained during the entire following twenty-four hours, but was most pronounced in the early hours of the day when it might be assumed that the concentration of these substances in the blood was highest. It is interesting to note that the total molecular concentration of the urine, as estimated by the freezing-point, was less on the day that sodium chlorid was added than on either the day preceding or that following. The electrical conductivity was nearly constant, but showed changes from hour to hour, and as might be expected averaged slightly lower on the days when the larger amount of sodium chlorid was excreted than on the control day. Meyer considers that such a reaction affords evidence of an inability of the kidney to increase its total concentrative powers. But as already pointed out by Meyer and Forschbach, the concentration per hundred c.c. of urine showed definite variations from hour to hour; so here again, according to Schlayer's views, is evidence of vascular hyposthenuria with hypersensitive vessels. The concentration of the urine varied, suggesting that the concentrative faculties of the kidney were still present, yet chlorid and urea acted, in proportion to their concentration in the blood, as diuretics on the irritable renal vessels. In other words, the sodium chlorid and urea acted on the kidney in much the same fashion as do intravenous injections of these diuretics in animals. Diuresis was most pronounced at first, and gradually diminished in ratio to a diminishing concentration in the blood.

Meyer[15] had already commented on the reaction of certain cases of diabetes insipidus to small doses of theocin. He found that this drug did not affect the total amount of fluid excreted, but increased the concentration of sodium chlorid in the urine so that during the twenty-four hours following its administration the excretion of salt was

increased. When theocin was omitted, the chlorid output diminished until the normal balance was regained. In this connection Mosenthal and Schlayer[33] have conducted interesting experiments in relation to renal fatigue by diuretics. They produced uranium or chromate nephritis in rabbits and studied the effect of repeated injections of sodium chlorid or caffein on the total output of water and salt, and on the volume of the kidney as recorded in an oncometer. In the early stage of chromate nephritis sodium chlorid produced an increased outflow of urine and salt with dilatation of the renal vessels, while subsequent injections of caffein caused a diminishing output of these substances with vascular contraction. In uranium nephritis, on the other hand, directly opposite results were obtained. Injections of sodium chlorid alone were ineffective in causing diuresis, while caffein, given later, reestablished the flow of urine. Mosenthal and Schlayer explained these findings on the assumption that in one type of lesion the kidney was fatigued by caffein with abnormal rapidity, and in the other type of lesion by sodium chlorid.

From the results of these studies it might be imagined that certain cases of diabetes insipidus with vessels "hypersensitive" to chlorid stimulation might be fatigued by the superposition of purin or other diuretics. In consequence, it might be expected that such diuretics would not increase the output of fluid, but might rather actually diminish it, and that the concentration of chlorid and its total output might be normal or even increased providing the tubules were capable of functional activity. The following experiments were made to test this hypothesis. On one day the patient was given 5 gm. of sodium chlorid; on another 0.6 gm. of theocin in divided doses; on a third, 5 gm. of sodium chlorid and 0.75 gm. of theocin, and on a fourth day 0.9 gm. of theobromin sodium salicylate (diuretin) in divided doses. The results of this treatment are shown in Chart 3.

It is seen that theocin and theobromin sodium salicylate alone had no diuretic action, nor did they increase the total output of sodium chlorid. In the presence of additional chlorid, theocin had no effect in preventing diuresis, but on the other hand it did not increase the difference between the intake and output of fluid and therefore seemed to have no indepeqdent action. It is interesting that when theocin and salt were given together, a larger total output of chlorid was obtained than when no theocin was included. The concentration was not increased, however, so no definite statement as to theocin effect can be made. Rather than to consider that theocin increased the output of salt, it is easier to believe the combination of drugs made the patient more thirsty than chlorid alone, and that a certain amount of excess

33. Mosenthal and Schlayer: Deutsch. Arch. f. klin. Med., 1913, cxi, 217.

salt was washed through the kidney in the large fluid bulk. These observations show definitely that, in our case at least, theocin superimposed on chlorid did not fatigue the hypersensitive renal vessels.

On the whole, these various tests for kidney function brought out several interesting features. According to Schlayer's views, the patient reacted to the differential tests for renal function as do cases of

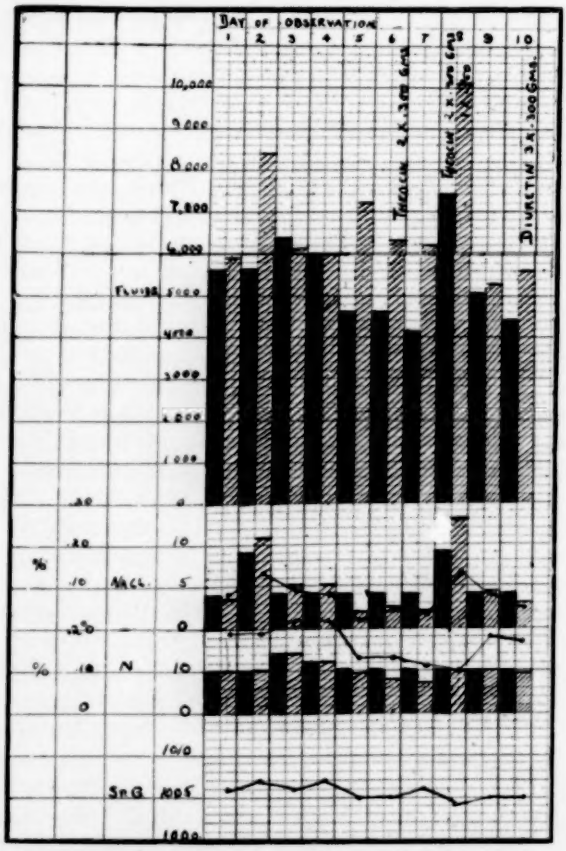


Chart 3.—The effect of diuretics on urinary output.

nephritis with "hypersensitive" vessels, but with normal renal function in other respects. There was a constant "vascular" hyposthenuria: "tubular" function was normal according to the iodid test and because the concentrative power of the kidney was not entirely lost. The excretion time of lactose was normal, as was to be expected on account

of the polyuria. These findings, however, justify the belief that no severe anatomical lesions existed in the kidney. This was confirmed by negative physical and urinary examinations, by a normal phenolsulphonephthalein elimination, and by a lack of rest-nitrogen accumulation in the blood.

There is insufficient evidence on which to draw any conclusions as to the underlying cause of the disease in this patient. Certainly, the renal findings are as well explained on the assumption of a lesion in a brain area without focal symptoms, which causes a hypersensitiveness of the renal vessels either by stimulation of the vasomotor nerves of the kidney or through hypersecretion of hypophyseal extract, as on the basis of a primary kidney disturbance. Either of these factors could produce a polyuria of the type encountered in which the renal vessels were hyperirritable to stimulation and in which the renal function was normal in other respects.

The treatment of diabetes insipidus has always been unsatisfactory. Certain cases of syphilitic origin have been reported which have shown improvement under the administration of iodids and mercury, but at least as many have been treated without benefit. Valerian has been advocated by the French school under Trosseau, but results from its use have been inconclusive. A gradual restriction of fluid intake has been suggested by Geigel,[34] Strubell,[35] Schwenkenbecher[22] and others. But this means has proved too uncomfortable for the patients to warrant its general usage. To illustrate the suffering of those patients whose fluids are limited, Trousseau[36] quotes the following case of a young man who was passing daily as much as 32 liters of urine.

> In the wards in which he was placed, he was subjected to an almost total abstinence from drink; and to quench his craving thirst he was given some ice or lemon to suck, allowing him to take food in accordance with the promptings of his appetite. The unfortunate young man resigned himself to this severe regimen, which he endured for eight months, although he suffered so cruelly from it that one day he seized the chamber-pot and drank the contents to the last drop.

Strubell's case is equally dramatic. The patient under observation was locked in a room with two iron-barred windows. Strubell kept the key. The patient, however, became so thirsty when his drink was cut down that once he captured a half liter of rain-water in a most complicated fashion, as it ran off the roof; once he drank the water in which he bathed, and once, 1,400 c.c. of his own urine. It is only fair to add that the recent work of Socin tends to show that a thirst cure may be borne without ill effect or too great discomfort.

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34. Geigel: Deutsch. Arch. f. klin. Med., 1885, xxxvii, 51.
35. Strubell: Deutsch. Arch. f. klin. Med., 1899, lxii, 89.
36. Trousseau: Lectures on Clinical Medicine, New Sydenham Society, London, 1870, iii, 530.

A more rational form of therapy is to treat the patient not only for syphilis if it exists, but also according to the excretory capacity of his kidney, using a bland and sufficiently nourishing diet which will not excite a violent polyuria. Intercurrent infection has been observed by many writers to have a beneficial effect on diabetes insipidus. Külz[8] and Pauli[37] have emphasized this feature in the disease, and Strubell[35] has tested it experimentally by the production of an artificial fever

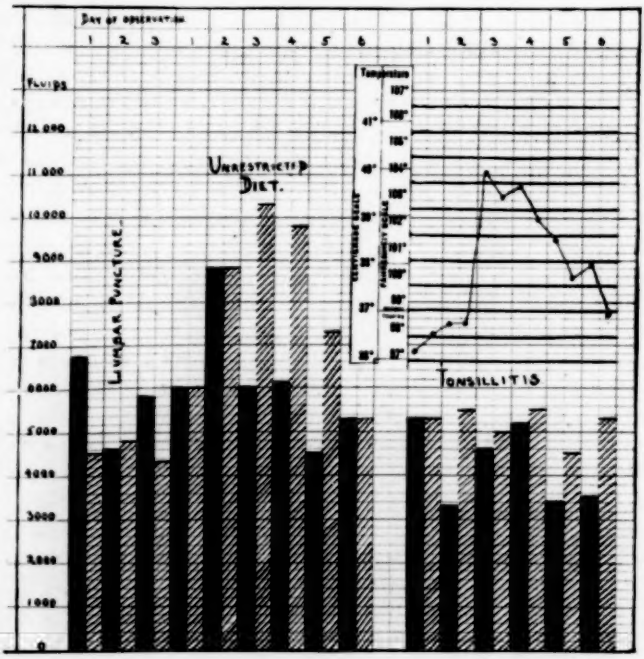

Chart 4.—The effect of lumbar puncture, of diet and of intercurrent fever on the output of urine.

from the subcutaneous injection of deutero-albumose. Finally, Herrick[38] has reported a case in which lumbar puncture seemed to relieve the symptoms strikingly. In our case, a month's antisyphilitic treatment has had no effect. The patient received four injections of salvarsan, and has been given mercury and iodid. At present his symptoms are unchanged, and the Wassermann reaction has not

37. Pauli, Inaug. Diss., Zürich, 1891.

38. Herrick, James B.: Report of a Case of Diabetes Insipidus with Marked Reduction in the Amount of Urine Following Lumbar Puncture, THE ARCHIVES INT. MED., 1912, x, 1.

diminished, although it is impossible to predict the eventual result of this form of treatment. The best results, therapeutically, have been obtained by giving the patient food which contained relatively small amounts of sodium chlorid and nitrogen. He was kept on a restricted diet for a considerable period, and, at last, as a reward, was allowed to eat anything he chose for three days. The results of this change of diet were so striking that the patient himself noticed them and asked to be given his former diet. From this it is evident that dietetic treatment may afford considerable relief to the symptoms. That acute infections and fever do not always benefit the disease was shown in this case. The patient developed an acute tonsillitis with fever for three and one-half days, during and immediately following which there was no definite change in the amount of urine excreted. Lumbar puncture, also, afforded no relief to the patient's symptoms. These facts are illustrated in graphic form by Chart 4.

SUMMARY AND CONCLUSIONS

Frank's original definition for diabetes insipidus still holds, "a long-continued abnormally increased secretion of non-saccharine urine which is not caused by a diseased condition of the kidneys." There are three much-discussed theories in regard to the pathologic physiology of the disease: (1) that it is due to faulty concentrative powers on the part of the kidney; (2) that it is a primary polydipsia with normal renal function; (3) that it is a symptomatic polyuria induced by stimulation of the kidney from a variety of causes, but in a certain number from hypersecretion of the hypophysis. It is a chronic disease not amenable to treatment. Since it is so frequently of syphilitic origin, syphilis should be treated when it is present. In other respects, each case must be treated individually, in accordance with the excretory capacity of the kidney, and with avoidance of substances which exaggerate the polyuria.

Tests for renal function in the case reported showed that the kidney as a whole was normal, but gave findings characteristic for vascular hyposthenuria with vessels hypersensitive to chlorid stimulation. Other diuretic substances and minute amounts of salt were inactive. The concentrative powers of the kidney were not entirely lost. This case therefore is probably not due to a primary lack of ability to concentrate on the part of the kidney. Variations in diet produced marked variations in both subjective and objective symptoms. Lumbar puncture and intercurrent fever had no visible effect on the disease, nor did one month's antiluetic treatment.

CONTRIBUTIONS TO THE EXPERIMENTAL PATHOLOGY OF THE STOMACH

I. EXPERIMENTAL PYLORIC STENOSIS *

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The sequence of events responsible for the clinical and pathological findings in disease of the stomach is often difficult if not impossible to analyze. The difficulty is directly proportional to the number and complexity of the processes involved. For example, in chronic gastric ulcer one attempts to analyze and properly coordinate the increase or decrease of gastric acidity, secretion, and motility; pyloric spasm or stenosis; dilated or hypertrophied stomach, etc. Through long clinical custom certain views are current as to the cause and effect of these interrelating processes, without much question as to the validity of these views or without subjecting them to experimental proof.

It has seemed to us that in view of the complexity of the problem of chronic ulcer of the stomach, these several processes should be separated and studied individually, a method, as Bolton[1] points out, which has not hitherto been adopted.

The following paper is presented as the first of a series of reports concerning the experimental pathology of the stomach and contains the results of producing primary partial stenosis of the pylorus in a series of twenty-nine dogs. It includes incidental reference to the relation of pyloric obstruction to chronic ulcer, a question which will be discussed at greater length in a subsequent paper.

METHODS

Small male and female dogs weighing from 6 to 8 kg. were used exclusively. Under usual surgical asepsis and complete surgical anesthesia an upper abdominal incision is made somewhat to the right of the mid line to insure easy access to the pyloric region. The stomach and antrum are pulled up into the wound and a fairly heavy silk ligature passed under the pylorus with the aid of an aneurysm needle and tied as near as possible to the duodenopyloric junction, avoiding the compression of large blood-vessels. The ligature is tied suffi-

* Submitted for publication June 4, 1914.
* From the Morris Institute for Medical Research.
1. Bolton: Proc. Roy. Soc. London, 1909-10, lxxxii, B 241.

ciently tight to produce permanent obstruction as evidenced by deep peristaltic waves in the antrum, cramp of the pylorus with temporary anemia, antiperistaltic waves, etc. Considerable experience is necessary to produce proper tension of the ligature, as in some instances it is tied too loosely, producing little, if any, obstruction, or too tightly, causing complete stenosis with death of the animal within from two to five days. The high mortality in many of our earlier dogs is to be attributed to this latter cause.

After experimenting with various test-meals and methods of introducing and withdrawing them, the following method was chosen as a routine: The dogs were fasted from eighteen to twenty hours over night and in the morning were given 100 gm. chopped lean beef and 300 gm. milk introduced by stomach-tube. In from fifty to sixty minutes 1/20 grain apomorphin hydrochlorid is injected hypodermatically, resulting in two to three minutes in the emesis of the test-meal, which is collected in a large evaporating dish. In the obstructed animals there is present, in addition to the meat and milk residue, large rests of beef, cartilage, bones, potatoes, carrot, etc. of food eaten days before. The apomorphin method possesses advantages over other methods of withdrawal by tube in obtaining total residue of stomach. The meals were examined for total quantity, free and total acidity, the finer chemical and microscopic examination being dispensed with as unessential.

In many of the dogs a second laparotomy was made in intervals of from ten days to three weeks, when 1 or 2 c.c. of 5 per cent. silver nitrate was injected at various points (pylorus, fundus, lesser curvature) producing acute ulcers. In four dogs (Nos. 11, 30, 31, 13) the pyloric ligature and injections were made simultaneously, resulting in all instances in complete obstruction and death of the animal. Two dogs (Nos. 44, 52) were injected daily with 1/5 grain pilocarpin hydrochlorid and 1/50 grain eserin sulphate respectively, to produce recurring stenosis from pyloric spasm.

RESULTS

The results of pyloric occlusion may be grouped in three divisions, dependent on the degree of obstruction.

GROUP 1.—Moderate stenosis (ligature tied loosely) resulting in little change in gastric acidity, motility or size (ten dogs—Table 1).

GROUP 2.—Marked obstruction (ligature tied sufficiently tight to cause marked peristalsis and anemia) resulting in definite change in motility (decrease) acidity (increase) and size (dilatation and hypertrophy) (nine dogs—Table 3).

GROUP 3.—Complete obstruction (from occluding ligature or edema from silver nitrate injection) resulting in inanition, vomiting, convulsions and death of the animal in from forty-eight to one hundred and twenty hours (ten dogs—Table 4).

Group 1.—This is perhaps the most interesting of the series, demonstrating the resisting power of the dog's stomach, as indicated by maintenance of normal acidity, motility, and size in the presence of a pyloric ligature tied for obstruction and producing at time of operation definite pyloric obstruction. For the most part these animals showed no loss in weight or cachexia, ate their food greedily

TABLE 1.—MODERATE—

Dog No.	Test Meal Before Operation			First Operation	Test Meal After First Operation		
	Date	Amount	Total Acid	Pyloric Ligature	Date	Amount	Total Acid
41	12/23	200	37	12/26	.....	.....	.....
51	2/5	200	51	2/6	2/17	350	54
35	.....	.....	.....	11/25	.....	.....	.....
36	.....	.....	.....	12/20	.....	.....	.....
42	.....	.....	.....	1/8	1/26	250	55
43	.....	.....	.....	1/15	1/26	450	26
7	.....	.....	.....	6/20	.....	.....	.....
6	.....	.....	.....	6/26	.....	.....	.....
26	.....	.....	.....	11/20	.....	.....	.....
28	.....	.....	.....	11/25	.....	.....	.....

without apparent discomfort, grew fat and sleek in spite of their occluding ligatures, and had every evidence of good care and health in marked contrast to their appearance on entrance into the laboratory.

Test-Meals: We found for the most part a slight increase in total quantity and total acidity within from ten to fourteen days after pyloric obstruction. Two dogs (51, 42) following a primary hyperacidity gave a secondary drop to normal within six weeks of the original operation. Necropsy in one to three months after ligation revealed a slightly or moderately dilated stomach, the latter occurring in those dogs whose test-meals had shown hyperacidity. In Dog 41, however, in spite of an increase from 37 to 52 total acid in two months, the stomach was found of normal size.

The control test-meal findings in dogs in which no preliminary meal has been given, were obtained from a series of six normal dogs as shown in Table 2:

Most of the animals were necropsied in approximately two months after ligation. In every instance the silk ligature was found in place,

buried usually in the serosa or muscularis coats and covered with connective tissue and adhesions, and adherent to the under surface of liver or gall-bladder.

Group 2.—In these animals, as a result of more securely tied ligature with marked pyloric obstruction, the changes of hyperacidity, hypersecretion, motor insufficiency, dilated and hypertrophied stom-

—Pyloric Stenosis

Second Operation	Test Meal After Second Operation			Necropsy			
$AgNO_3$	Date	Amt.	Total Acid	Date	Stomach	Ulcer	Scar
.....	2/13	425	25	2/28	Small	No ulcer	No scar
2/19	3/17	200	30	3/20	Small	No ulcer	No scar
12/18	1/26	150	38	2/20	Small slight hypertrophy	Two small healing ulcers	
12/30	1/26	350	26	2/20	Slightly dilated	Chronic pyloric ulcer	
.....	2/29	325	36	3/8	Slight dilatation	No ulcer	No scar
2/13	2/24	550	50	3/23	Slightly dilated; slight hypertrophy.	Chronic pyloric ulcer	
.....	.....	.....	.....	7/26	Fundus dilated; pyloric hypertrophy.		
.....	.....	.....	.....	7/10	Slight dilatation and hypertrophy		
12/13	.....	.....	.....	12/27	Small	Two acute ulcers	
12/10	.....	.....	.....	12/30	Slight dilatation	Both ulcers healing	

TABLE 2.—Test-Meal Findings in Six Normal Dogs

Dog No.	Total Quantity	Free Acid	Total Acid
1	150 c.c.	0	30
2	250 c.c.	0	31
3	350 c.c.	0	27
4	150 c.c.	0	25
5	200 c.c.	0	35
6	100 c.c.	0	32
Range—100-350 c.c. Average—200 c.c.		Range—25-35 Average—30	

ach were more marked. The dogs appeared ill at ease, grew thin and emaciated, refused food as if in pain, later became toxic and unable to stand, finally dying in from three weeks to two months from inanition and toxemia.

Test-Meals: A marked increase in total quantity up to one liter of contents (five times normal) much of this being food residue

but more supernatant fluid (hypersecretion). In all a marked primary hyperacidity was found, many showing later a secondary relative decrease (hypacidity) the total quantity remaining the same or

TABLE 3.—MARKED—

Dog No.	Test Meal Before Operation			First Operation	Test Meal After First Operation		
	Date	Amount	Total Acid	Pyloric Ligature	Date	Amount	Total Acid
55	2/5	200	44	2/12	2/24	950	56
38	1/14	250	26	1/23	2/2	550	36
50	2/5	300	59	2/6	2/18	250	75
48	.....	.....	.....	1/15	1/26	450	26
39	.....	.....	.....	1/16	1/26	800	62
37	.....	.....	.....	12/18	.....	.....	.....
22	.....	.....	.....	10/9	.....	.....	.....
21	.....	.....	.....	10/8	.....	.....	.....
5	.....	.....	.....	6/5	6/26	100	23

TABLE 4.—COMPLETE—

Dog No.	Date	Operation	Postoperative Condition and Course
4	5/27	Pyloric ligature	Sudden death in 72 hours with convulsions
2	5/20	Pyloric ligature	Constant vomiting, gradual inanition
9	7/17	Pyloric ligature	Progressive inanition. Death in 96 hours
17	9/4	Pyloric ligature	Sudden death in 96 hours with convulsions
19	9/11	Pyloric ligature	Sudden death in 48 hours
24	11/27	Pyloric ligature and $AgNO_3$	Sudden death in 72 hours
13	8/22	Pyloric ligature	Sudden death in 96 hours
31	12/4	Pyloric ligature and $AgNO_3$	Sudden death in 96 hours
30	12/4	Pyloric ligature and $AgNO_3$	Sudden death in 48 hours
11	8/6	Pyloric ligature and $AgNO_3$	Sudden death in 72 hours

becoming higher. Number 48 showed a late hyperacidity of 50 without previous increase.

Group 3.—The animals in this series died more or less suddenly in from forty-eight to 120 hours. A few refused food, vomited what

little water they drank and succumbed from progressive inanition and weakness. Most of the dogs were found dead in their cages in the morning, having been in good condition the previous evening,

—Pyloric Stenosis

Second Operation	Test Meal After Second Operation			Necropsy		
$AgNO_3$	Date	Amt.	Total Acid	Date	Stomach	Ulcer
2/26	3/24	1050	42	4/13	Markedly dilated	Chronic pyloric ulcer
2/13	.....	.....	.....	2/26	Markedly dilated	No ulcer or scar
2/19	3/20	600	64	3/21	Markedly dilated and hypertrophied	Superficial pyloric ulcer
2/13	2/24	550	50	3/23	Slightly dilated—Marked hypertrophy	Two duodenal ulcers—two chronic pyloric ulcers
.....	2/24	525	62	2/28	Markedly dilated and hypertrophy	No ulcer or scar
12/27	1/26	350	50	2/20	Markedly dilated	Chronic pyloric ulcer
10/30	.....	.....	.....	11/20	Markedly dilated	Unhealed pyloric ulcer, healing lesser curv. ulcer
.....	.....	.....	....	10/30	Markedly dilated and hypertrophy	
7/17	.....	.....	.....	8/21	Markedly dilated	Chronic pyloric ulcer

—Pyloric Stenosis

Necropsy			
Date	Stomach	Pylorus	Stomach Contents
5/30	Slightly dilated	Complete obstruction	Moderate amount clear fluid
5/25	Markedly dilated	Edema of all coats	Glairy mucus covering fundus
7/21	Small	.....	Small amount thick mucus
9/8	Small	Tightly contracted	50 c.c. thin milky fluid
9/13	Small	Strongly contracted	Mucoid fluid in stomach
11/30	Small	Thick and edematous	Four acute ulcers
8/26	Small	.....	Small amount frothy mucus
12/6	Small	Edema of antrum	
8/9	Small	Firmly contracted	Little fluid in stomach

eating their food, standing and walking about the cage, etc. Two of them (Nos. 4 and 16) were seized suddenly with tonic convulsions resembling tetany and died within five minutes. The condition of these dogs suggests the clinical picture of gastric tetany seen occasion-

ally in benign pyloric obstruction and is in accord with the experimental findings of Kaufman,[2] MacCallum[3] and others.

At necropsy the pyloric antrum is tightly contracted as if in tonic spasm, the fundus slightly dilated, the stomach of usual size, containing a small amount of thin fluid and glairy mucus. In one case (No. 2) in which there had been constant vomiting, with progressive inanition and loss in weight, the animal living for five days, the stomach was quite markedly dilated.

DISCUSSION

The interpretation of the motor insufficiency, continuous secretion, hyperacidity, hypertrophy and dilatation of stomach produced by pyloric obstruction, we believe to be the following: As a result of the narrowed pyloric canal, food normally propelled into the duodenum within from six to twelve hours is retained twenty-four hours or even several days. During this time the pylorus is in a state of continuous peristaltic activity in attempts to drive the contents, through the obstruction, on into the intestines—a prolongation and intensification of the normal peristalsis described by Cannon.[4] The grinding and churning of the food caused by this increased peristalsis presses the gastric contents with increased violence against the pyloric mucosa, stimulating the production of a chemical stimulant by the gastric mucosa, which Edkins[5] has shown is responsible (after its absorption into the blood) for the continued secretion of gastric juice. We have found that as late as forty-four hours after test-meals, the obstructed dog's stomach contained large quantities of contents with high free (34) as well as total (90) acidity, a condition never seen in normal dogs. We conclude, therefore, that the hyperacidity resulting from obstruction is really a hypersecretion (continuous secretion) produced as described and persists as long as food is present in the stomach. The histology of hyperacidity will be reported on later.

The explanation of the hypertrophy and dilatation is not so clear, some dogs with marked obstruction having small stomachs with hypertrophied walls, others having marked dilatation, while dogs with only slight obstruction have very markedly dilated stomachs. In all probability the gastric muscle tonus plus a possible subacute or chronic gastritis are additional factors, although it may be entirely a muscle affair analogous to the response of the heart to a stenosis. Micro-

2. Kaufmann: Am. Jour. Med. Sc., 1908, cxxvii, 606.
3. MacCallum: Zentralbl. f. allg. Path., 1905, xvi, 385.
4. Cannon: Mechanical Factors of Digestion, 1911, Chapter VI. Effect of Stomach Movements.
5. Edkins: Jour. Physiol., 1906, xxxiv, 133.

scopic examination of the stomach wall is necessary to substantiate this. The results will be reserved for later publication.

In the dogs with *successful* partial pyloric obstruction (motor insufficiency, continuous secretion) the acute ulcers produced by silver nitrate injection were in many instances prevented from healing, or became chronic and progressive, particularly the pyloric ulcers (see Bolton). We believe that the constant mechanical trauma of the retained food, grinding forcibly back and forth on the raw surface, plus the continuous corrosive action of free hydrochloric acid (the stomach never being free from acid) are two of the most important factors in the production of chronic experimental ulcer.

CONCLUSIONS

1. Moderate pyloric stenosis in dogs (ligature around pylorus tied loosely) causes little or no change in stomach motility, acidity or size.

2. Marked obstruction (ligature tied sufficiently tight to produce marked peristalsis and anemia of the pylorus) causes motor insufficiency, continuous secretion (hyperacidity) and hypertrophy and dilatation of the stomach.

3. Complete occlusion (ligature or silver nitrate injection causing secondary occluding edema) results in inanition, vomiting, convulsions (gastric tetany) and death of the animal in from forty-eight to 120 hours.

4. The pyloric ligature (stenosis) results in a motor insufficiency with food retention which in turn sets up a continuous secretion with the constant presence of free hydrochloric acid.

5. Pyloric obstruction with resulting motor insufficiency and continuous secretion are probably the most important factors in the production of *chronic* experimental ulcer.

6. While the foregoing results concern *experimental* chronic ulcer, it is more than likely that primary partial pyloric stenosis (from whatever cause) with secondary motor insufficiency and continuous secretion are largely responsible for the progression and delayed healing of chronic ulcer in man.

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A CLINICAL AND BACTERIOLOGICAL STUDY OF HEXAMETHYLENAMIN AS A URINARY ANTISEPTIC *

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The increasing use of hexamethylenamin for pathological conditions has led to more systematic studies of its chemical and bactericidal properties, and, as a result, recent literature abounds with papers on the subject. Following the discovery by Nicolaier,[1] in 1894, that hexamethylenamin was broken down into formaldehyd in the urine, this drug was used extensively in infections of the genito-urinary tract. Up to 1902 the literature contained many papers on its use, a good review of which may be found in the paper by Lubowski.[2] For a few years after 1902 very little new work was done on hexamethylenamin as an antiseptic agent until 1908, when Crowe[3] demonstrated its presence in body-fluids other than the urine. Since that time there has been a revival of interest in this drug and its uses, and as a result it has become more or less a panacea for many ills to which the body is heir.

The findings of several of the recent workers not being entirely in harmony, and hexamethylenamin being used to a large extent in this hospital, it was deemed of interest to learn from personal experience in what conditions it is indicated. It was through the courtesy of the attending physicians of this hospital that permission was granted to use cases from their services and this opportunity is taken to thank them for their kindness and advice. Thanks are also due to the director of the laboratory for the use of laboratory records and material.

As Nicolaier[1] and others, Cammidge,[4] Citron,[5] Hanzlik and Collins,[6] Hinman,[7] Jordan,[8] Sollmann,[9] Talbot and Sisson,[10] Smith,[11] Vinde-

* Submitted for publication June 9, 1914.

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1. Nicolaier: Centralbl. f. d. Med. Wissensch., 1894, xxxii, 897.
2. Lubowski: Therapist, 1904, xiv, 114, 131, 151, 165.
3. Crowe: Bull. Johns Hopkins Hosp., 1908, xix, 109.
4. Cammidge: Lancet, London, 1901, ii, 174.
5. Citron: Monatschr. u. d. Krankh. d Harn- u. Sexualapp., 1898, iii, 73.
6. Hanzlik and Collins: Hexamethylenamin: The Liberation of Formaldehyd and the Antiseptic Efficiency Under Different Chemical and Biologic Conditions, THE ARCHIVES INT. MED., 1913, xii, 578.
7. Hinman: An Experimental Study of the Antiseptic Value in the Urine of the Internal Use of Hexamethylenamin, Jour. Am. Med. Assn., 1913, lxi, 1601.
8. Jordan: Biochem. Jour., 1911, v, 279.
9. Sollmann: Formaldehyd Derivatives, Jour. Am. Med. Assn., 1908, li, 818
10. Talbot and Sisson: Boston. Med. and Surg. Jour., 1913, clxviii, 485.
11. Smith: Boston Med. and Surg. Jour., 1913, clxviii. 713.

vogel,[12] have shown, the value of hexamethylenamin as a therapeutic agent depends on its decomposition into formaldehyd in acid mediums. That alkalies may also decompose hexamethylenamin into formaldehyd has been mentioned by few, Reché,[13] Jenness,[14] Sollmann.[9] This last worker, however, states that hexamethylenamin is only very slightly acted on by alkalies. From the nature of the chemical reaction this does not seem possible.

The reaction illustrates a true reversible equation as follows:

$$(CH_2)\ 6N_4 + 6H_2O \rightleftarrows 4NH_3 + 6CHO$$

Hexamethylenamin + water Ammonia + formaldehyd

Inasmuch as this is a true reversible equation, the law of mass action would apply and the direction of the reaction would depend on the proportion of the reagents used. Hence, the least amount of acid which really acts as a hydrolyzing substance when added to hexamethylenamin will cause the reaction to go toward the formaldehyd end, and this has been shown very well by those who have used weak acids, such as 0.2 per cent. hydrochloric acid (Hanzlik and Collins[6]), dilute sodium acid phosphate and beta-oxybutyric acid (Smith[11]). In this present work 1 c.c. of a tenth-normal hydrochloric acid solution has been used and good tests for formaldehyd have been obtained. It has also been repeatedly shown that a neutral solution of hexamethylenamin itself, on being boiled or incubated, will break down into formaldehyd. This is undoubtedly due to the hydrolyzing action of the water. On the other hand, Hinman[7] and Hanzlik and Collins[6] have shown that alkalies added to hexamethylenamin prevent its decomposition. In testing out the bactericidal action of hexamethylenamin, as will be shown later, its decomposition could always be prevented by the addition of enough ammonium hydroxid to give it a concentration of 1:40,000. After incubating this solution, tests for formaldehyd were always negative. As a still further proof that formaldehyd is never present free in alkaline mediums after the administration of hexamethylenamin, may be mentioned the fact that it is never found in the alkaline fluids of the body. In the face of the foregoing facts it is difficult to explain the action of hexamethylenamin, or rather its derivative formaldehyd, in any but acid mediums.

There is, however, another phase to the question which must not be overlooked and which has not been dwelt on sufficiently. It has been known that both hexamethylenamin and formaldehyd combine with other substances found in the various fluids of the body. Nicolaier[15] mentions the fact that hexamethylenamin unites with uric acid to form

12. Vindevogel: Ann. soc. Roy. d. sc. med. Bruxelles, 1902, li, 20.
13. Reché: Inaug. Dissert., Breslau, 1902.
14. Jenness: Burnam's Test for Formaldehyd in the Urine, Jour. Am. Med. Assn., 1913, lx, 662.

a very soluble diformaldehyd uric acid. Citron[5] has stated that the bactericidal or inhibitory action is not due to formaldehyd, but to a sodium compound of it. Kobert[16] says that hexamethylenamin breaks down into ammonia and formaldehyd and forms with insoluble urates, soluble salts of formaldehyd uric acid. Hammarsten[17] and Cammidge[4] both state that when formaldehyd is added to urea a combination takes place. The reaction between formaldehyd and albumin is well known in the use of formaldehyd as a hardening substance. Schwarz[18] has shown that formaldehyd in combination with albumin forms methylene-albuminate combinations. Therefore, the question arises whether the favorable results obtained in some mediums, especially alkaline mediums, are not due to some of these compounds of hexamethylenamin or formaldehyd which may be inhibitory or bactericidal. Such combinations would mask the tests for free formaldehyd. There is still the possibility that fluids which are normally alkaline, in pathological conditions may become just acid enough to liberate free formaldehyd, which immediately combines with the increased amount of albumin present forming a new compound in itself, also either inhibitory or bactericidal. The ammonia liberated from the hexamethylenamin at the same time that the formaldehyd is formed, would neutralize the acid present and the fluid would remain neutral or alkaline. This would be one theory to explain the beneficial action of hexamethylenamin in some cases of meningitis in which the organisms present are acid-producing organisms. It would be of interest to study the bactericidal actions of some of these compounds of hexamethylenamin or formaldehyd.

There is hardly a secretion or fluid in the body in which hexamethylenamin after administration is not found. It can always be demonstrated in urines of those using the drug, in the saliva (Hanzlik,[19] Crowe,[2] Burnham[20]), in the middle ear and nasal sinuses (Barton,[21] Leibecke[22]), in the bronchi (Heitmueller,[23] Leibecke[22]); in the aqueous and vitreous humors of the eye (Whitman,[24] Hanzlik and Collins[6]), in

15. Nicolaier: Arch. f. klin. Med., 1904, lxxxi, 181.
16. Kobert: Lehrbuch der Intoxicationen, 1906, ii, 1116.
17. Hammarsten: Text-Book of Physiol. Chem., 1908, p. 556.
18. Schwarz: Ztschr. f. physiol. Chem., 1900, xxxi, 36.
19. Hanzlik: The Excretion of Hexamethylenamin in the Saliva, Jour. Am. Med. Assn., 1910, liv, 1940.
20. Burnham: An Experimental Investigation of the Value of Hexamethylenamin and Allied Compounds, THE ARCHIVES INT. MED., 1912, x, 324.
21. Barton: Bost. Med. and Surg. Jour., 1910, clxii, 896.
22. Liebecke: Berl. klin. Wchnschr., 1913, l, 1698.
23. Heitmueller: Excretion of Hexamethylenamin, Jour. Am. Med. Assn., 1910, liv, 1223.
24. Whitman: Jour. Ophth., 1912, xli, 604.

tears (Gradle[25]); in synovial fluids (Crowe,[3] Hanzlik and Collins[6]); in pleural fluids (Hanzlik and Collins,[6] Shattuck[26]); in pericardial fluids (Hanzlik and Collins[6]); in peritoneal fluids (Leibecke[22]); in pancreatic juice (Crowe[3]); in bile (Crowe,[3] Burnham[20]); in milk (Leibecke,[22] Crowe,[3] Hanzlik and Collins,[6] Rieder,[27] Buchura,[28] Schmidt and Schroter[29]); in eruptions on the body (Sachs[30]); in lochia (Hanzlik and Collins[6]); in cerebrospinal fluids (Burnham,[20] Crowe,[3] Flexner and Clarke,[31] Hald,[32] Hanzlik and Collins,[6] Ibraham,[33] Leibecke[22]), and in blood-serum (Hald,[32] Vindevogel[12]).

In this hospital some indications for the use of hexamethylenamin have been:

1. As an antiseptic in infections of the genito-urinary tract such as pyelitis, cystitis, pyonephrosis and infections following calculi.
2. As a prophylactic preceding catheterizations.
3. As a urinary antiseptic in typhoid fever.

In this work the investigations have been confined to the examination of urines in those cases mentioned above. The urines in all cases have been examined for the presence of hexamethylenamin, formaldehyd, and for the amount of acidity. The use of the Rimini[34] test by Burnham[20] afforded an easy method for the recognition of formaldehyd and at the same time the results obtained could be used in comparison with those of others. The test consists of the use of three solutions:

1. Phenylhydrazin hydrochlorid, a 0.5 per cent. aqueous solution, 3 drops.
2. Sodium nitroprussid, a 5 per cent. solution, 3 drops.
3. Sodium hydroxid, saturated solution, 2 or 3 drops.

These solutions are added to from 5 to 10 c.c. of urine. In comparing the colors obtained with solutions of formaldehyd of known concentrations the results agree with Smith,[11] who found that in solutions of 1:10,000 or stronger, a blue color is obtained, 1:15,000 to 1:25,000 a greenish black, and weaker solutions, an intense green.

The acidity was determined by litmus paper and by titrating 10 c.c. of urine with tenth-normal sodium hydroxid solution using phenol-

25. Gradle: Ophth. Rec., 1911, xx, 110.
26. Shattuck: Boston Med. and Surg. Jour., 1911, clxiv, 842.
27. Rieder: Monatschr. f. Kinderheilk., 1912, xi, 80.
28. Bucura: Ztschr. f. exper. Path. u. Therap., 1907, iv, 398.
29. Schmidt and Schroter: Zentralbl. f. d. Physiol. u. Path. d. Stoffwechsel., v, 129.
30. Sachs: Wien. klin. Wchnschr., 1912, xxv, 153.
31. Flexner and Clark: Experimental Poliomyelitis in Monkeys, Jour. Am. Med. Assn., 1911, lvi, 585.
32. Hald: Arch. f. exper. Path. u. Pharmakol., 1911, lxiv, 329.
33. Ibraham: Med. Klin., 1910, xxxvi, 1893.
34. Rimini: Ann. di Farmacol., 1897, xvii, 97.

phthalein as an indicator. The litmus paper did not always agree with the acidity shown to phenolphthalein, especially when the titration required from 0.3 to 0.6 c.c. of tenth-normal sodium hydroxid solution. In these cases the litmus was either neutral or faintly alkaline. The acidity of the urines expressed in terms of 100 c.c. of urine ranged from 3 to 144. It was found that the acidity of the urines obtained within eight hours following an operation was usually high, from 50 to 80, one, 195, a few as low as from 30 to 40. It was also found that catheterized urines from patients not operated on ranged between 60 and 70. The average urine required from 3 to 3.5 c.c. of tenth-normal sodium hydroxid to neutralize 10 c.c. of urine. This agrees with Hinman,[7] who found the average to be 3.1 c.c.

The urines from 91 patients were examined. The greater number of these patients received 7 gm. of hexamethylenamin three times a day. Most of the urines were examined twice and some of them as many as six times. About 250 urines were examined. The 91 cases may be divided as follows: surgical 42, medical 32, genito-urinary 17. The urines of all of the 91 patients gave a test for formaldehyd varying from 1:20,000 to 1:5,000. The urines from 72 patients showed the presence of formaldehyd in the strength of 1:10,000. These results agree with those of Smith,[11] Talbot and Sisson,[10] Hanzlik and Collins,[6] Hinman,[7] all of whom found formaldehyd in over 97 per cent. of acid urines in patients using hexamethylenamin. Burnham,[20] L'Esperance,[35] Jenness[14] found formaldehyd in about 50 per cent. of their cases. In explanation of this fact Burnham proposed the theory that there is a selective action on the part of renal cells toward the breaking down of hexamethylenamin. Hinman[7] has disproved this by catheterizing the ureters of 23 patients, testing these urines and also the urines obtained at the same time from the bladders. Only 5 ureteral urines showed formaldehyd. Of the bladder urines only 3 failed to show formaldehyd. Somewhat similar results were found in 3 of the 91 cases reported in this paper. In these the ureteral urines were negative for formaldehyd, whereas the urines from the bladder showed its presence. In those cases in which formaldehyd is liberated in the kidneys it is probably due to the higher than normal acidity of the urine in the kidneys. These are undoubtedly the cases that develop a hematuria or albuminuria. It may be concluded, therefore, that in almost all cases hexamethylenamin is excreted into the bladder unaltered and then, under the influence of the acid in the urine and the warmth of the body, is broken down into formaldehyd.

35. L'Esperance: Bost. Med. and Surg. Jour., 1912, clxvii, 577.

The laboratory records of 100 urines, catheterized under aseptic conditions, show that the following organisms had been isolated:

Bacillus coli	64
B. pyocyaneus	11
B. proteus	4
B. paratyphosus	1
B. typhosus	1
B. mucosus capsulatus group	2
Staphylococcus aureus	8
S. citreus	1
S. pyogenes	6
B. fecalis alkaligenes	2
Total	100

These were recovered in the following cases:

Pyelitis	59
Pyonephrosis	12
Neprolithiasis	7
Cystitis	10
Miscellaneous	12

In this series of cases the colon bacillus was the most common invader of the urinary tract, occurring in 64 per cent. of the cases. To be of real therapeutic value, the hexamethylenamin or its derivative would have to be excreted in a concentration strong enough to be either inhibitory or bactericidal. Considerable work has been done on the antiseptic value of hexamethylenamin and formaldehyd. Stern[36] mentions patients with cystitis and pyelitis who had received hexamethylenmain for a long time and from whose urines he had isolated either the *Bacillus coli*, the *B. lactis aerogenes*, *B. pyocyaneus*, or the *Staphylococcus aureus*. Richardson[37] was one of the first to show that the *B. typhosus* disappeared very quickly from the urine of typhoid patients. Horton-Smith[38] and Biss[39] also found this to be true.

Experiments have been carried on in which the urines of patients who had received large doses of hexamethylenamin were inoculated with different organisms. Forcart[40] inoculated these urines with *B. coli*, *B. typhosus*, *B. proteus*, *B. pyocyaneus*, streptococcus, *Staphylococcus albus* and *S. aureus*. These were incubated for five days. He found that the colon bacillus was the most resistant.

Others have tested the inhibitory or bactericidal power of solutions of hexamethylenamin. The solutions have been added to cultures or emulsions of bacteria and incubated, or the solutions have been inoculated and incubated. It must be borne in mind that hexamethylenamin by itself when incubated or warmed gently will liberate a small amount

36. Stern: München. med. Wchnschr., 1910, lvii, 2273.
37. Richardson: Jour. Exper. Med., 1899, iv, 14.
38. Horton-Smith: Brit. Med. Jour., 1900, Pt. 1, 827.
39. Biss: Edinburgh Med. Jour., 1902, New Series, xii, 337.
40. Forcart: Med. Klin., 1908, iv, 335.

of formaldehyd and in those experiments in which weaker concentrations of hexamethylenamin were inhibitory or bactericidal it may have been this small amount of formaldehyd that produced the effect. The decomposition of hexamethylenamin, as has already been stated, may be prevented by the smallest amount of alkali.

Keuthe[41] states that 1:10,000 solution of hexamethylenamin inhibits the growth of *B. typhosus* while it requires a 1:3,000 solution to inhibit the *B. coli*. No mention is made of the possible action of formaldehyd which may have been liberated. Muller[42] dissolved hexamethylenamin in bouillon in the concentration of 1:1,000, and inoculated this with *B. coli, B. typhosus, Staphylococcus aureus* or *B. pyocyaneus*. These were incubated and there was no inhibition of growth. Ascitic fluid which was inoculated with *B. coli* was also treated with a 1 per cent. solution of hexamethylenamin and incubated. There was no inhibition at the end of forty-eight hours. Goetzl and Salus[43] found a 5 per cent. solution bactericidal for *B. typhosus, B. coli, B. proteus* and staphylococci. Cammidge,[4] on the other hand, found that although a 7.5 per cent. solution killed completely the typhoid bacillus that the same solution only inhibited the growth of *B. coli* and *Staphylococcus aureus*. Results are different when an alkali is first added to a solution of hexamethylenamin to prevent its decomposition into formaldehyd and then this solution is inoculated and incubated. Burnham[20] inoculated 5 per cent. and 10 per cent. solutions of hexamethylenamin containing a slight amount of ammonium hydroxid with *B. coli, B. typhosus, B. pyocyaneus*, streptococcus and *Staphylococcus aureus*. These were incubated and all of the organisms survived. Hanzlik and Collins[6] inoculated agar mediums containing sodium carbonate or ammonium hydroxid and hexamethylenamin with bacteria and found that it was not bactericidal. Agar mediums containing hexamethylenamin and no alkali showed no growth when the concentration of the hexamethylenamin was 1:50 or 1:100. In weaker concentration there was growth.

The action of a 10 per cent. solution of hexamethylenamin was tried here on various organisms. To some of these solutions ammonium hydroxid was added so that there was a concentration of 1:40,000 of ammonium hydroxid. To others no ammonium hydroxid was added. For a control, sterile saline containing 1:40,000 ammonium hydroxid was used. All of the solutions were inoculated with equal amounts of a twenty-four-hour culture of one of the following bacteria: *B.*

41. Keuthe: Inaug. Diss., Heidelberg, 1902; quoted in Centralbl. f. Bakteriol., 1902, xxxii, 379.
42. Muller: Deutsch. Aerzte. Ztschr., 1903, viii; quoted in Centralbl. f. Bakteriol., 1902, xxxiii, 808.
43. Gotzl and Salus: Ztschr. f. klin. Med., 1902, xlv, 427.

typhosus, B. coli, B. paratyphosus, B. acidi lacti, B. lactus aerogenes, B. pyocyaneus, streptococcus and *Staphylococcus aureus.* These were incubated for twenty-four hours at 37 C. (98.6 F.) and a subculture taken. The results are given in Tables 1, 2 and 3.

TABLE 1.—ACTION OF TEN PER CENT. HEXAMETHYLENAMIN WITH NO AMMONIUM HYDROXID ON GROWTH OF ORGANISMS *

	Reaction	Formaldehyd	Bacteria
Staphylococcus aureus	N	1:20,000	Growth
Bacillus typhosus	V. F. A.	V. F.	Growth
B. coli	V. F. A.	V. F.	Growth
B. paratyphosus	N	1:10,000	No Growth
B. acidi lactis	N	1:10,000	No Growth
B. lactis aerogenes	N	None	Growth
B. pyocyaneus	N	V. F.	Growth
Streptococcus pyogenes	N	V. F.	Growth
Control Hexamethylenamin....	N	V. F.	—
Control Hexamethylenamin + NH_3	S. A.	V. F.	—

* In this and the following tables, N = neutral; V. F. = very faint; F. V. A. = very faintly alkaline; S. A. = slightly alkaline.

It will be seen from the tables that incubating has effected a hydrolysis of those solutions of hexamethylenamin to which no ammonium hydroxid had been added. It may also be seen that the growth of only two of the organisms was inhibited by the small amount of formaldehyd liberated. This inhibition was present at the end of twenty-four

TABLE 2.—ACTION OF TEN PER CENT. HEXAMETHYLENAMIN, CONTAINING 1:40,000 AMMONIUM HYDROXID ON GROWTH OF ORGANISMS

	Reaction	Formaldehyd	Bacteria
Staphylococcus aureus	S. A.	0	Growth
Bacillus typhosus	S. A.	0	Growth
B. coli	S. A.	0	Growth
B. paratyphosus	S. A.	V. F.	Growth
B. acidi lactis	S. A.	V. F.	Growth
B. lactis aerogenes	S. A.	0	Growth
B. pyocyaneus	S. A.	0	Growth
Streptococcus pyogenes	S. A.	0	Growth

hours. There was growth at the end of forty-eight hours. Similar experiments were carried on with bouillon containing 10 per cent. of hexamethylenamin and inoculated with *B. typhosus* or *B. paratyphosus.* Subcultures taken after twenty-four hours' incubation showed growth in all.

It may readily be seen that hexamethylenamin *per se* even up to a 10 per cent. solution has neither bactericidal nor marked inhibitory properties. Since hexamethylenamin is never present in the urine in concentrations as great as 10 per cent. it must be concluded that when an inhibitory or bactericidal action is shown in the urine it cannot be due to the hexamethylenamin.

TABLE 3.—ACTION OF SALINE CONTAINING 1:40,000 AMMONIUM HYDROXID ON GROWTH OF ORGANISMS

	Reaction	Bacteria
Staphylococcus aureus	S. A.	Growth
Bacillus typhosus	S. A.	Growth
B. coli	S. A.	Growth
B. paratyphosus	N	Growth
B. acidi lactis	N	Growth
B. lactis aerogenes	S. A.	Growth
B. pyocyaneus	S. A.	Growth
Streptococcus pyocyaneus	S. A.	Growth

From the work of Burnham,[20] Hess,[44] Kolle and Wassermann,[45] Kendall and Edwards,[46] Hanzlik and Collins,[6] Hinman,[7] Table 4 may be deduced.

TABLE 4.—CONCENTRATIONS OF HEXAMETHLENAMIN REQUIRED TO INHIBIT GROWTH AND TO KILL, RESPECTIVELY, IN TWENTY-FOUR HOURS

	To Inhibit	To Kill
Bacillus typhosus	1:15,000	1:5,000
B. coli	1: 7,000	1:1,000
B. pyocyaneus	1: 7,000	1:1,000
Streptococcus	1: 5,000	1:1,000
Staphylococcus aureus	1: 5,000	1:1,000

Formaldehyd to be of value must be liberated in strengths varying from 1:15,000 to 1:5,000, depending on the organism the growth of which is to be inhibited. To be of bactericidal value it must be liberated in strengths from 1:5,000 to 1:1,000, also depending on the organism. There is no simple chemical test for determining the exact amount of formaldehyd in urine. An approximate estimation may be made by diluting the urine with water in various proportions and testing these different proportions according to the Rimini method. By such a method formaldehyd was never demonstrated in a concentration greater than 1:5,000. Hence it is reasonable to assume that formaldehyd is probably never present in urine in solution strong

44. Hess: Quoted by Borner, Centralbl. f. Bakteriol., Orig., 1910, liii, 415.
45. Kolle and Wassermann: Handbuch. der pathogene Mikroorgamismen, ii, 387; iii, 115.
46. Kendall and Edwards: Jour. Infect. Dis., 1911, viii, 250.

enough to destroy any organism except the *B. typhosus*. It may, however, have an inhibitory action on other organisms.

The acidity factor must also be considered. There is still some question as to whether or not there is a relationship existing between the amount of acid present and the amount of formaldehyd liberated. It has been shown by several that formaldehyd may be liberated by the smallest amount of free acid. Jordan[8] states that the percentage of formaldehyd, and with it the antiseptic power, increases with the acidity until the acidity has reached slightly above normal. He also

TABLE 5.—Acidity of Urine, Amount of Formaldehyd Liberated and Resulting Action on Bacteria

	Time Examined	Acidity	Formaldehyd	Bacteria
Patient A—				
After Voiding				
Minutes	30	64	1: 5,000	Sterile
Hours	24	51	1: 5,000	Sterile
After Inoculation, hrs.	24	38	1: 10,000	Growth
Patient B—				
After Voiding				
Minutes	30	68	1: 5,000	Sterile
Hours	24	62	1: 10,000	Sterile
After Inoculation, hrs.	24	62	1: 10,000	Growth
Patient C—				
After Voiding				
Minutes	30	62	1: 10,000	Sterile
Hours	24	60	1: 10,000	Sterile
After Inoculation, hrs.	24	50	1: 10,000	Sterile
Patient D—				
After Voiding				
Minutes	30	10	1: 20,000	B. coli
Hours	24	Alkaline	1: 25,000	
After Inoculation, hrs.				
Patient E—				
After Voiding				
Minutes	30	28	1: 20,000	B. coli;
Hours	24	10	1: 20,000	S. albus

states that when it reaches this point the urine remains sterile. To determine if there is any relation existing between the acidity, the amount of formaldehyd liberated and the resulting action of the two on bacteria the following experiments were conducted:

Patients who had been given hexamethylenamin over a period of time were catheterized as aseptically as possible. The acidity and amount of formaldehyd was determined soon after voiding, and again twenty-four hours after the urine had been incubated. Cultures of these urines were taken and those which were found sterile were inoculated with cultures of *B. coli*. These were incubated again for twenty-

four hours, and the acidity, amount of formaldehyd determined and subcultures taken. The results are given in Table 5.

In the two cases in which the acidity is low, that is, 10 and 28, the strength of formaldehyd found is less than in the urines of higher acidity. In these two cases the urines were collected through permanent catheters and the continuous flow probably accounts for the lower acidity. In three other urines in which the acidity was greater than normal the growth of the colon bacillus was only inhibited in one. The formaldehyd in this case had a concentration of 1 : 10,000. It may be concluded, therefore, that there is a relationship between the amount of acid present and the amount of formaldehyd found, and that in urines whose acidity is much higher than normal the combination of the increased acid and the formaldehyd may either inhibit or prevent the growth of bacteria other than the *B. typhosus*.

TABLE 6.—Effect of Increasing Acidity of Normal Sterile Urine After Twenty-Four Hours

	Acidity	Formaldehyd	Culture
1.—Normal urine	21		
2.—Normal urine, *B. coli*.....	8	.....	Growth
3.—10 c.c. normal urine, 7.5 per cent. hexamethylenamin..	8	1 : 20,000	
4.—10 c.c. normal urine, 7.5 per cent. hexamethylenamin, *B. coli*	8	1 : 20,000	Growth
5.—10 c.c. normal urine, acidity increased to 55, 7.5 per cent. hexamethylenamin, *B. coli*	20	1 : 10,000	Growth
6.—10 c.c. normal urine. Acidity increased to 80, 7.5 per cent. hexamethylenamin, *B. coli*	27	1 : 10,000	No Growth
7.—7.5 per cent. hexamethylenamin, *B. coli*.....	Neutral	Negative	Growth

The effect of increasing the acidity was attempted in another way. Normal sterile urine of known acidity was used and the experiments shown in Table 6 tried. The acidity of this urine was 26. The amount of acidity was increased by adding tenth-normal hydrochloric acid.

Growth was inhibited in a urine with an initial acidity of 80 and a final formaldehyd strength of 1 : 10,000.

The acidity of urines of patients who had been using hexamethylenamin for some time was increased by the addition of a calculated amount of tenth-normal solution of hydrochloric acid. These were inoculated with the colon bacillus and incubated. The acidity in both cases was made up to 60.

In these two cases an acidity of 60 and a final formaldehyd strength of 1 : 10,000 was inhibitory. In these cases in which hydrochloric acid was added and growth was inhibited it must be borne in mind that the kind of acid used might also have been an important factor in causing the inhibition of growth. This is a reasonable assumption when we consider that a solution of formaldehyd of a concentration of 1 : 10,000 is not strong enough by itself to inhibit the growth of *B. coli*. It would, therefore, seem that the ideal method of treatment would be to increase the acidity of the urine to at least twice the normal by appropriate means at the same time that the hexamethylenamin is given. The only acid or acid salt that seems best adapted to this purpose is acid sodium phosphate. This, if pushed too hard, has its complications, the most common being diarrhea. It must also be borne in mind that the urinary tract is not a test-tube, and, whereas there are no dangers of destruc-

TABLE 7.—Effect of Increasing Acidity of Patients Who Had Used Hexamethylenamin for Some Time

	Time Examined	Acidity	Formaldehyd	Bacteria
Patient F.....				
After Voiding	Immediately	46	1 : 10,000	
	24 hours	44	1 : 10,000	
After Inoculation	24 hours	42	1 : 10,000	No growth
Patient G.....	Immediately	52	1 : 20,000	
After Voiding	24 hours	47	1 : 10,000	
After Inoculation	24 hours	40	1 : 10,000	No growth

tion to tissue in a highly acid urine when the experiments are conducted in a test-tube, there may be considerable injury done when the experiment is carried on through the kidney. This is especially true when the formaldehyd is liberated in the kidney tubules or pelvis through the action of a highly acid urine. The large number of recorded cases of hematuria and albuminuria is a proof of this fact.

SUMMARY

1. Hexamethylenamin, *per se*, in neutral solutions in concentrations up to 1 : 10 is neither inhibitory nor bactericidal.

2. Hexamethylenamin when given in doses of 7.5 grains three times a day is broken down into formaldehyd in all acid urines.

3. Formaldehyd is formed in the bladder in all cases except in those in which the acidity of the urine is higher than normal. In these cases some formaldehyd is formed in the kidneys.

4. The common invader of the urinary tract is the colon bacillus and the strength of formaldehyd necessary to inhibit its growth is a concentration of 1 : 5,000 or greater.

5. By testing different dilutions of urine it has been found that with a dose of 7 grains of hexamethylenamin three times a day, formaldehyd is never present in concentrations greater than 1 : 5,000.

6. The only organism which is destroyed or whose growth is inhibited by this concentration is the typhoid bacillus.

7. For the destruction or inhibition of growth of organisms other than the typhoid bacillus a high acidity is necessary. This high acidity in combination with the formaldehyd may produce injury to the kidney tissue.

8. Hexamethylenamin appears most efficacious as a prophylactic during the course of typhoid fever for the prevention of a pyelitis or cystitis. Its use in this connection has been demonstrated successfully both clinically and bacteriologically.

BLOOD-PLATELETS AND TUBERCULOSIS*

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So little is known concerning blood-platelets, and so little interest has been taken in them, that their description by Adami and Nichols[1] is almost fully quoted.

If only because they play so important a part in the process of thrombosis it is necessary to have a clear understanding regarding the blood-platelets, or, more accurately, regarding what is known concerning their origin.

Apart from this, since the advent of the Romanowsky stain and its modifications, they have of late years come in for increasing recognition.

There is no longer any disposition to regard them as artefacts, but there is still dispute as to their exact significance and as to their unity or duality.

They are small bodies of varying size, in general about 2 μ in diameter, oval or pear-shaped, evidently labile, and varying in shape with slight compression by neighboring cells or platelets, tending to be present in smears in small groups (possibly as the result of rapid agglutination in shed blood).

They are non-nucleated although containing often fine, central granules, which assume a redder tint with the Romanowsky stain in contrast to the bluer ground work.

Pratt's careful studies show that they are present in the normal blood in greater numbers than the leukocytes, although the number shows wider variations from 200,000 to 700,000 per c.mm.

Since their discovery and the early papers by Hayem, Bizzozero and Mrs. Ernest Hart, there have been very various views regarding their nature and mode of origin.

1. That they are precipitated globulin (Löwit. Woolbridge).
2. That they are products of disintegration of white corpuscles (Lilienfeld and Zenker).
3. That they are given off from disintegrating red corpuscles (Mono, Klebs and Arnold).

The exquisite preparations made by J. H. Wright of Boston, demonstrate without possibility of doubt that some at least of the platelets, and those most typical, are normally derived from a particular order of cell, namely from the giant cells (megakaryocytes) of the bone-marrow.

These cells give off processes projecting into the lumina of the capillaries, and it is the distal portions of these which become liberated into the blood as platelets. As such they may, as Schimmelbusch was the first to demonstrate, retain some power of ameboid movement, but the mode of their development sets at rest the debate as to the nature of the central staining granules, they in no sense represent a nucleus. . . .

We must admit that in pernicious anemia the platelets are frequently (though not always) diminished in number, and that here there has been observed a lack of giant cells in the bone-marrow.

Submitted for publication June 18, 1914.

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1. Adami and Nichols: Principles of Pathology, 1911.

In purpura they have at times been found completely absent. We know of no observations on the marrow giant cells in these cases. They are diminished also in typhoid, but increased in myelogenic leukemia and in pneumonia.

Port and Akijama[2] regard as normal 230,000 to 240,000 blood-platelets per cubic millimeter, with slight daily variations.

In acute infections (pneumonia, typhoid, scarlet fever) they found diminished numbers during the fever, but a large increase coincident with the drop in temperature. In chronic diseases they found very slight typical changes.

They think the blood-platelets play a part in the immunity processes as carriers of protective substances which are set free on their destruction.

Pagniez[3] doubts the necessity of platelets being present for blood coagulation, pointing out that lymph will coagulate without these elements. He reviews all the present knowledge of blood-platelets and suggests that some functions attributed to leukocytes may belong to the platelets, pointing out how difficult it is to procure leukocytes free from platelets.

Werbitzki[4] found that the blood of rabbits and horses contained antibodies for anthrax bacilli, which are different from serum bacteriolysin and the bactericidal substances of leukocytes, and which he traced to the platelets, but he found that the blood-platelets of the ox, hog, dog and man had no such substance. Werbitzki determined also that the blood-platelets of the above-mentioned animals and man had no bactericidal effect on typhoid bacilli, colon bacilli, staphylococci or the bacilli of dysentery or cholera.

Eminet[5] has demonstrated that blood-platelets in different infectious diseases possess different and specific affinities for stains.

He calls the blood-platelets *Soterozytes*, and shows in infection by the diphtheria bacillus, for instance, that these elements do not stain as they do in tuberculosis or scarlet fever cases, and vice versa. He considers therefore that the blood-platelets share in specific immune processes.

Grüber and Futaki[6] showed that the lymph or serum of a rabbit which has been heated to 65 C. (149 F.) can be given strong bactericidal power against anthrax bacilli if mixed with well-washed blood-pletelets (obtained by injecting sterile water under the skin of the rabbit).

2. Port and Akijama: Klinische Untersuchungen über Blutplattchen, Arch. f. klin. Med., cvi, Nos. 3 and 4.
3. Pagniez: Arch. d. mal. du coeur, d. vaisseaux et due sang, January, 1909.
4. Werbitzki: Ztschr. f. Hyg. u. Infections-krankh., 1911, lxviii, No. 1.
5. Eminet, Charkow: Arch. f. Kinderh., lvii, Nos. 4 and 6.
6. Gruber and Futaki: München. med. Wchnschr., 1907, No. 6.

Blood-plasma of rabbits and rats is completely without action against these bacilli. They believe the bactericidal power of the serum arises from a substance given to it by the platelets during the coagulation of the blood. This substance given up by the platelets is not thermolabile alexin, but really the same as is found in the serum of rabbits and rats. (Ottolenghi comes to another opinion.)

The platelet extract in physiological salt solution is not bactericidal, and the platelet extract in citrated blood-plasma is not active. (Ottolenghi says this is because the blood-platelets remain unchanged in this fluid and complement will only be freed when they undergo change as in the formation of fibrin or when they are mixed with serum or lymph.)

Blood-platelets were found to increase the bactericidal power of the normal serum of the rabbit, probably because the normal serum has many amboceptors, but not sufficient complement to activate all of them.

In studying the bactericidal power for anthrax bacilli of the leukocytes, Grüber and Futaki found those taken from "congestion lymph" more powerful than others.

The living plasma of the normal rabbit was found to be entirely free from antagonistic power to anthrax bacilli. The protective body gets into the serum only when the blood clots.

They conclude that the bactericidal substance furnished by the platelets to the serum is entirely different from the thermolabile alexin.

Ottolenghi[7] described the presence of complement in fibrin and found he could reactivate inactive serum and bring back the bactericidal power against certain bacteria by fibrin or its extract in salt solution.

Schneider[8] first demonstrated the method of procuring pure platelets by fractional centrifugation. He also demonstrated the connection between platelets and the formation of fibrin and blood-clotting.

Schneider also showed the possibilities of reactivating serum with an extract of platelets.

Aynaud[9] found that animals sensitized with platelets will react with red corpuscles and vice versa.

Aynaud[10] has noted the high amount of platelet content which is soluble in the solvent of fats, and also has noted the presence of phosphorus. He thinks these fats agree with the peculiar staining qualities of the platelets and their insolubility in the gastric juice, and their

7. Ottolenghi: München. med Wchnschr., 1907, No. 17.
8. Schneider: München. med. Wchnschr., 1907, No. 3.
9. Aynaud: Compt. rend. Soc. de Biol., 1911, No. 37.
10. Aynaud: Presse méd., April 1, 1914.

digestion by the pancreatic juice lend argument in favor of a diffused nuclear substance in the element.

Wells[11] says: The blood-platelets are believed to be largely nucleoproteid, but little is known of their actual composition.

It is still undetermined just what part the platelets play in coagulation. Pratt, however, found that the number of platelets bore no relation to the coagulability of the blood, and that lymph which is free from platelets will coagulate.

Welch describes the steps in the formation of a thrombus after injury to the vessel-wall, as follows: First, there is an accumulation of blood-platelets adhering to the wall at the point of injury. Leukocytes which may be present in small numbers at the beginning, rapidly increase in number, collecting at the margins of the platelet masses and between them. Not until the leukocytes have accumulated does the fibrin appear.

TABLE 1.—Blood-Platelet Count of Dr. A. Taken at 12 Noon, at Colorado Srrings

Date	Platelets	Leukocytes
4/22/13	216,000	
4/23/13	184,000	
4/25/13	228,000	
4/26/13	184,000	
4/28/13	216,000	
4/29/13	288,000	6,000
4/30/13	222,000	6,000
5/ 1/13	180,000	6,200
5/ 2/13	228,000	6,800
5/ 3/13	168,000	5,800
5/ 5/13	228,000	6,000
5/ 6/13	220,000	6,000
5/ 8/13	222,000	6,000
5/12/13	164,000	6,000
5/14/13	248,000	6,000
5/15/13	208,000	5,400
5/17/13	244,000	5,800
5/20/13	180,000	6,200

The work to be presented consists of (1) a series of platelet counts in normal adults at sea-level and at an altitude of 6,000 feet; and a series in tuberculous patients and tuberculous animals at various stages, and (2) several series of experiments relative to the possible functions of blood-platelets.

The estimation of blood-platelets per cubic millimeter has until recently been most inexact on account of the lack of suitable technic. In the beginning of this work the metaphosphate method of Pratt was followed.

11. Wells: Chemical Pathology, 1907, p. 264.

All the counts below enumerated, however, were made according to the technic described by Wright and Kinnicutt.[12] Two parts of cresyl blue (1:300) are mixed with three parts of potassium cyanid (1:14,000) and rapidly filtered, then immediately used to dilute the blood 1:100. The blood must be from a free-flowing drop and the whole procedure must be carried out as quickly as possible.

The method appears reliable judging by the closeness of counts reported for the same individual.

The counts on Dr. A., a normal individual, given in Table 1, show the accuracy of the method which compares favorably with the accuracy of the successive enumerations of the white corpuscles.

Tables 2 and 3 give the counts of blood-platelets on two hundred normal male adults between the ages of 20 and 25, taken at sea-level (New York) and at an altitude of 6,000 feet (Colorado Springs). All the observations were made at 12 noon, when no digestion was occurring and no unusual exercise had been indulged in.

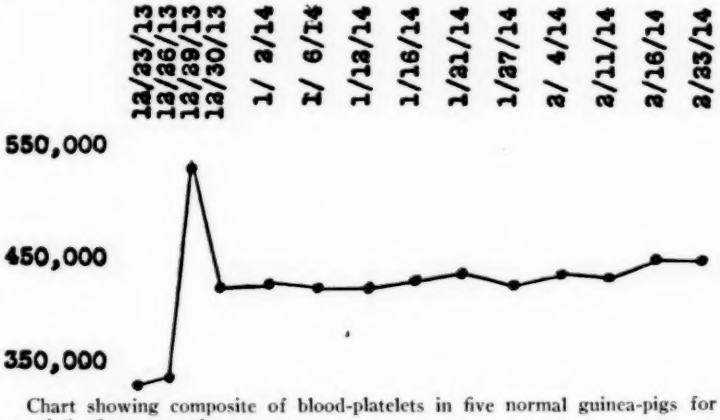

Chart showing composite of blood-platelets in five normal guinea-pigs for a period of two months.

The New York counts were made for us by Dr. Carolyn Rosenberg under the direction of Dr. T. W. Hastings of the Cornell Medical School. Dr. Gilbert and Dr. Rosenberg carefully went over the technic of counting together in New York.

These counts give an average of 302,000 blood-platelets at sea-level and 340,000 at an altitude of 6,000 feet. This is a percentage increase of 12.2.

The accompanying composite chart shows the average numbers of blood-platelets in five normal guinea-pigs immediately after arrival in

12. Wright, James Homer, and Kinnicutt, Roger: A New Method of Counting the Blood-Platelets for Clinical Purposes, The Journal A. M. A., May 20, 1911, p. 1457.

TABLE 2.—Blood-Platelet Count Made on Two Hundred Normal Male Adults in New York and Colorado Springs

No.	Cornell Students Platelet Count	Colorado College Students Platelet Count	No.
64	458,000	484,000	18
34	442,000	456,000	47
7	426,000	448,000	20
35	426,000	444,000	41
36	426,000	416,000	86
66	426,000	404,000	91
100	410,000	404,000	48
49	410,000	404,000	56
61	410,000	396,000	78
88	410,000	392,000	61
79	398,000	388,000	64
27	394,000	388,000	1
42	394,000	388,000	84
48	394,000	384,000	62
52	394,000	384,000	44
67	394,000	384,000	52
95	378,000	384,000	88
93	378,000	384,000	68
16	378,000	380,000	71
24	378,000	376,000	11
28	378,000	372,000	74
51	378,000	372,000	50
62	378,000	372,000	31
71	378,000	368,000	7
78	352,000	368,000	8
20	352,000	368,000	15
3	352,000	368,000	25
12	352,000	368,000	27
74	352,000	368,000	59
91	325,000	368,000	73
80	336,000	364,000	26
73	336,000	364,000	42
2	336,000	364,000	83
9	336,000	360,000	39
11	336,000	360,000	57
47	336,000	360,000	58
57	336,000	360,000	80
65	336,000	360,000	89
84	320,000	356,000	93
69	320,000	352,000	63
55	320,000	352,000	77
32	317,000	352,000	95
77	304,000	348,000	87
72	304,000	348,000	94
68	304,000	348,000	100
21	304,000	344,000	70
23	304,000	340,000	5
29	304,000	340,000	25
50	304,000	340,000	90
82	304,000	336,000	2
1	288,000	336,000	13
6	288,000	336,000	49
22	288 000	332 000	22

TABLE 2.—(*Continued*)

No.	Cornell Students Platelet Count	Colorado College Students Platelet Count	No.
30	288,000	332,000	65
38	288,000	328,000	14
59	288,000	328,000	17
75	288,000	328,000	55
85	288,000	328,000	79
89	288,000	328,000	99
96	288,000	324,000	28
99	272,000	324,000	45
70	272,000	324,000	67
60	272,000	324,000	97
4	272,000	320,000	3
8	272,000	320,000	12
37	272,000	320,000	76
97	256,000	320,000	81
90	256,000	316,000	4
83	256,000	316,000	6
56	256,000	316,000	19
53	256,000	316,000	72
45	256,000	312,000	10
14	256,000	312,000	33
18	256,000	312,000	38
25	256,000	312,000	51
33	240,000	312,000	82
43	240,000	312,000	96
58	240,000	308,000*	43
26	240,000	304,000	9
54	240,000	304,000	75
92	240,000	300,000	21
40	224,000	300,000	32
63	224,000	300,000	35
98	224,000	292,000	69
5	224,000	292,000	66
87	224,000	292,000	24
10	224,000	288,000	36
15	224,000	288,000	46
19	224,000	288,000	85
44	208,000	284,000	29
13	208,000	284,000	98
31	208,000	276,000	16
46	208,000	272,000	37
81	192,000	272,000	53
41	192,000	268,000	54
94	192,000	268,000	92
39	192,000	252,000	30
76	176,000	252,000	34
86	176,000	252,000	60
17	160,000	240,000	40
	Average, 302,000 Mean... 288,000	Average, 340,000 Mean... 336,000	Pct. Inc., 12.2

Colorado Springs from sea-level and at intervals for two months thereafter.

We[13] have shown elsewhere that blood-platelets were also increased in the circulating blood by (1) carbon monoxid poisoning, (2) hyperemia of the marrow of long bones.

It has been known for some time that blood-platelets were increased in tuberculosis. Our observations both in guinea-pigs and man agree with this.

Other blood-elements are increased as in Table 3.

Tables 4 and 5 show a series of counts on healthy and tuberculous guinea-pigs.

It is especially worthy of notice that not only was a distinct increase in the leukocytes recorded in the tuberculous guinea-pigs, an average of 13,200 per cubic millimeter, as against an average of 7,620 per cubic millimeter for healthy pigs, but also a decided gain in the number of blood-platelets from an average of 438,000 per cubic millimeter in healthy pigs to an average of 622,000 in the tuberculous animals.

TABLE 3.—OTHER BLOOD-ELEMENTS IN NEW YORK AND COLORADO

	Sea Level Percentage	Colorado Springs Percentage	Per Cent. Increase
Lymphocytes	36	43	19.3
Hemoglobin	100	110	10.0
Red Corpuscles	5,000,000	6,000,000	20.0

We have divided the counts on tuberculous patients into the following classes:

1. Early afebrile tuberculosis, 45 counts; 35 patients; 368,000 blood-platelet average.

2. Advanced quiescent tuberculosis, 50 counts; 40 patients; 412,000 blood-platelet average.

3. Advanced active tuberculosis, 26 counts; 22 patients; 496,000 blood-platelet average.

The observations on children with measles, given in Table 6, are of considerable interest.

It is at once seen that a decrease of platelets takes place during the disease.

Only further observations can determine whether the low platelet count may be connected with the high incidence of tuberculosis after measles.

13. Webb, Gilbert and Havens: Colorado Med., January, 1914.

TABLE 4.—Blood-Platelet Count in Normal Guinea-Pigs at Colorado Springs, Colo.

No.	Date	Platelets	Total Leukocytes
1	8/ 4/13	454,000	9,800
2	.....	476,000	8,000
3	.....	428,000	6,400
4	.....	444,000	7,600
5	8/ 5/13	444,000	7,000
6	8/ 7/13	464,000	9,000
7	.....	384,000	7,200
8	8/ 8/13	368,000	6,200
9	.....	392,000	7,000
10	8/12/13	482,000	8,000
11	2/ 4/14	424,000	
12	2/ 4/14	476,000	
13	2/ 4/14	408,000	
14	2/ 4/14	464,000	
15	2/ 4/14	364,000	
16	2/11/14	432,000	
17	2/11/14	412,000	
18	2/11/14	424,000	
19	2/11/14	440,000	
20	2/16/14	464,000	
21	2/16/14	484,000	
22	2/16/14	436,000	
23	2/16/14	424,000	
24	2/23/14	448,000	
25	2/23/14	424,000	
26	2/23/14	528,000	
		Average 434,000	Average 7,600

TABLE 5.—Blood-Platelet Count in Tuberculous Guinea-Pigs at Colorado Springs, Col.

No.	Date	Platelets	Total Leukocytes
1	7/24/13	696,000	14,000
2	7/24/13	760,000	13,000
3	7/25/13	724,000	12,000
4	7/25/13	604,000	11,000
5	7/25/13	576,000	12,000
6	8/12/13	612,000	11,000
7	8/12/13	776,000	10,000
8	8/13/13	492,000	11,000
9	8/13/13	540,000	11,000
10	8/13/13	596,000*	15,000
11	8/13/13	468,000*	12,000
		Average 622,000	Average 13,200

*Counts 10 and 11 were made direct from jugular vein under ether. All others were made by cutting the pig's ear.

It is conceded that Nature rarely operates without a purpose. Callus is not thrown out, as a rule, unless a bone is broken. In pneumonia we have generally a remarkable increase in the numbers of the polymorphonuclear leukocytes, the reason for which is better understood since Metchnikoff's studies in phagocytosis.

It has been recognized for some time that in several diseases, including tuberculosis, the blood-platelets become remarkably increased in number.

It is reasonable, therefore, to ask why do these elements increase, and do they take any part in immunity phenomena?

The microscope has never revealed their presence in the structure of tubercles, the formation of which is considered an immunity process. But other immunity processes are at work in the defense of the organism against the tubercle bacillus, for we know of the production of agglutinin, opsonin and, possibly, lysin and other immune bodies.

TABLE 6.—Blood-Platelet Count in Children Ill with Measles

Case No.	Days Since Rash Appeared	Platelet Count
1	4	248,000
1	14	520,000
2	5	292,000
2	16	348,000
3	3	260,000
3	7	300,000
4	1	288,000
4	3	276,000
5	5	260,000
6*	4	290,000

*An adult tuberculous patient. The tuberculosis pursued a rapid course following this attack of measles.

It is natural to ask, then, if Nature increases the blood-platelets in the victim of tubercle infection; is it for a purpose comparable with that found by Grüber and Futaki in rabbits or rats infected with anthrax?

Metchnikoff has maintained that antibodies, such as opsonins, are set free from leukocytes when these cells disintegrate. It is thought that serum as opposed to blood-plasma is very rich in opsonins, yet the work of Dastre[14] proves that the white corpuscles are not disintegrated when blood coagulates.

The thought occurred, therefore, can platelets furnish opsonin? The experiments shown in Table 7, several times carefully and thor-

14. Dastre, quoted by Aynaud: Le globulin de l'homme, Ann. de l'Inst. Pasteur, January, 1911.

oughly repeated, certainly suggest that they can. The table presents a composite picture of the results of five careful and complete experiments.

Similar experiments were carried out with similar results with staphylococci, so that it is not impossible that blood-platelets contain or supply opsonin.

For these and the subsequent experiments, the platelets were obtained by fractional centrifugation in citrated normal salt solution. It was well known to those who worked with the opsonic index that the fluid remaining after throwing down the corpuscles by means of the centrifuge, contained platelets. This fluid was now removed by a pipet and centrifuged at a high speed. A faint sediment was thrown down and the supernatant fluid withdrawn. This sediment was twice washed and centrifuged with normal salt solution. The final sediment was found to consist of almost entirely pure blood-platelets. We have pointed out[13] elsewhere that vagaries in the opsonic index might partly depend on the amount of platelets the washed corpuscles contained.

TABLE 7.—Composite Results of Authors' Experiments with Blood-Platelets and Tubercle Bacilli

	Polymorpho-nuclears	Large Mono-nuclears	Percentage of Empty Cells	
			Polymorpho-nuclears	Large Mono-nuclears
Serum*	1.05	0.83	26	29
Thick platelet emulsion ...	1.41	1.33	18	20
Salt solution*	0.59	0.47	37	41

* With tubercle bacilli emulsion and white corpuscues.

Experiments were undertaken in June, 1913, to ascertain if the addition of platelets to tubercle bacilli might prevent infection when inoculated simultaneously into a guinea-pig. The culture employed was later found to be dead and so the tests were worthless.

We have shown repeatedly that the subcutaneous inoculation of a definite number of very virulent bacteria (50 tubercle bacilli of a culture from which 10 bacilli have been found to cause infection), isolated by the Barber technic, will almost invariably cause infection, and such infection is always accompanied first by enlargement and infection of the adjacent lymph-nodes. We have made it a rule to inoculate always into the nipple area so that subsequent enlargement of the groin glands can be readily detected; usually after the second week.

It has frequently happened with these small doses that no local lesion has developed, but in animals so inoculated we have so far never missed the glandular involvement when the pigs have later been found tuberculous at necropsy.

The following experiments would, therefore, seem unusual:

Four young guinea-pigs were inoculated in the right nipple area.

Pig 1, weight 255 gm., received 200 tubercle bacilli.

Pig 2, weight 212 gm., received 200 tubercle bacilli + platelets from normal pig after incubation one-half hour.

Pig 3, weight 214 gm., received 200 tubercle bacilli.

Pig 4, weight 183 gm., received 200 tubercle bacilli + platelets from normal pig after incubation one-half hour.

Air was blown in following the injection in all except Pig 3. Forty-two days later Pig 1 was killed. No local tuberculous lesion; glands enlarged in right groin; extensive infection of spleen; mediastinal glands infected; extensive disease in liver. Thirty-four days later Pig 2 died of epidemic intestinal disease. One caseous nodule was found in the spleen. Nothing else was found, no local lesion and no groin glands infected. Forty-four days later Pig 3 was killed. No disease was found. It is probable that the bacteria were not safely inoculated. Forty days later Pig 4 died. No local lesion. No local glands in groin involved; extensive tuberculous infection in liver and spleen and mediastinal gland enlarged.

The chief interest of this experiment would seem to be in the absence of infection of the groin glands in Pigs 2 and 4.

This absence of local glandular infection is most unusual and possibly might suggest a sensitizing of the bacilli by the platelets allowing a more general distribution in the system.

We have found in earlier experiments that 60 bacilli of this virulent culture will produce rapid and wide-spread lesions in such young guinea-pigs as well as infection of the glands adjoining the site of inoculation.

The following experiments deal with older guinea-pigs, usually full-grown. The injection in all animals was made in the right nipple area.

Dec. 29, 1913: Pig 53; 200 tubercle bacilli. Pig 54; 200 tubercle bacilli with human platelets. Pig 55; 200 tubercle bacilli with human platelets and inactive serum. Pig 56; 200 tubercle bacilli with active (human) serum.

Jan. 18, 1914: Pig 55 has enlarged hard gland right groin. Others negative.

Feb. 16, 1914: Pig 56 has enlarged hard gland right groin.

April 11, 1914: Pig 55, killed. Enormous tuberculous liver and spleen; lungs completely involved (local lesion and glands had been previously removed).

April 23, 1914: Pig 53 gave birth to two young three weeks ago. Killed; no tuberculosis discovered. It is probable the bacilli were not safely inoculated.

April 23, 1914: Pig 56 gave birth to two young two weeks ago. Killed. Tuberculous spleen, few tubercles in liver, local gland, mediastinal gland.

April 17, 1914: Pig 54 has not yet shown infection. It is possible that bacilli were not safely inoculated. Fifty bacilli of same culture were now inoculated into left nipple area and also into a control, Pig 86.

May 23, 1914: Pig 54, killed. Spleen markedly tuberculous, bronchial gland and deep inguinal gland, left side affected. No local lesion. Pig 86, killed. Spleen and bronchial glands affected. No local lesion.

This last experiment indicates that if the 200 bacilli were originally safely inoculated, immunity was not produced sufficiently to protect the pig later against 50 tubercle bacilli.

The foregoing experiments were repeated with the same virulent culture, but instead of human blood-platelets, "immune" platelets from another pig and serum of this pig were employed.

Jan. 12, 1914. Pig 57, weight 565 gm.; 200 tubercle bacilli injected. Pig 58, weight 555 gm.; 200 tubercle bacilli injected, + platelets taken from a guinea-pig with advancing tuberculosis. Pig 59, weight 437 gm.; 200 tubercle bacilli injected; serum unheated; platelets taken from a guinea-pig with advancing tuberculosis. Pig 60, weight 513 gm.; 200 tubercle bacilli; serum heated to 55 C. for one-half hour; platelets taken from a guinea-pig with advancing tuberculosis.

All the pipets were incubated with their contents one-half hour before inoculations were made.

Feb. 7, 1914: Pig 60, large gland palpable in right inguinal region.
Feb. 12, 1914: Pig 57, large gland palpable in right inguinal region.
Feb. 14, 1914: Pig 59, large gland palpable in right inguinal region.
Feb. 16, 1914: Pig 58, no enlarged glands palpable.
April 3, 1914: Pig 57, local inguinal glands stationary or smaller. Pig 58, no local enlarged glands. Pig 59, local glands increasing in size. Pig 60, local glands very large.
April 8, 1914: Pig 57, found dead; extensive tuberculosis of liver and spleen; large mediastinal glands; local glands had diminished in size recently and no tuberculosis was found.
April 25, 1914: Pig 59, killed. Extensive tuberculosis of spleen; moderate infection of lungs and liver; mediastinal glands very large; local inguinal glands enlarged.
April 25, 1914: Pig 60, killed. Moderate tuberculosis of spleen. Scattered nodules in lungs and liver. Mediastinal glands very large. Local inguinal glands palpable.
May 16, 1914: Pig 58, found dead. No tuberculous lesion anywhere. Death due to epidemic gastro-enteritis. It is, of course, again possible that the bacilli were not safely inoculated.

We realize that not enough experiments have yet been carried out and that the work is incomplete. We may, however, consider that:

1. It is suggestive that when a small amount of a thick emulsion of platelets is added to a lethal dose of virulent tubercle bacilli, infection would seem to be either modified or prevented.

2. When serum is added to this mixture, no evidence can be shown of any change in the infection produced from that of the controls.

CONCLUSIONS

1. Blood-platelets are consistently increased in the tuberculosis of man and guinea-pigs.

2. At an altitude of 6,000 feet, blood-platelets are increased in the circulating blood of man and guinea-pigs.

3. Blood-platelets would appear either to contain or to supply opsonin.

4. Blood-platelets added to a lethal dose of tubercle bacilli appear to modify the course of infection or to prevent it.

5. The addition of serum to a mixture of blood-platelets and tubercle bacilli appears to interfere with this modification.

The foregoing work was aided by a grant from the American Medical Association to Dr. Gerald B. Webb.

Our grateful thanks are due Mr. and Mrs. B. C. Allen and Mr. Richard F. Howe for generous financial assistance.

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PULMONARY PHYSICAL SIGNS AND ROENTGEN-RAY FINDINGS IN HEALTHY ADULTS*

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The general campaign against tuberculosis, and particularly the influence of tuberculosis sanatoriums, has kindled an eager interest in the early diagnosis of pulmonary tuberculosis. Treatment, to be availing, must be begun before the presence of moist râles in the lungs and tubercle bacilli in the sputum proclaims the destruction of tissue. The various biological tests, hypersensitiveness, complement fixation, agglutination, etc., that have lent such valuable aid in the diagnosis of other infectious diseases have failed in tuberculosis, because in tuberculosis, contrary to the conditions in other infections, infection and disease are not synonymous terms. Therefore, effort has been directed toward refining fundamental diagnostic methods. The symptomatology of tuberculous disease has been more thoroughly analyzed and many valuable symptomatic relations have been established. It is only exceptionally, however, that a diagnosis can be made from the symptoms alone, since similar symptoms and symptom-groups occur in many other conditions. In most instances a satisfactory diagnosis is reached only when symptoms are supported by the presence of pulmonary physical signs.

The physical examination having become the diagnostic arbiter in nearly all doubtful cases of pulmonary tuberculosis, the arts of percussion and auscultation have been cultivated assiduously and to-day are practiced with heretofore unequalled precision. Slight lesions are discovered with remarkable accuracy as control Roentgen-ray examinations have demonstrated.

ABNORMAL PHYSICAL SIGNS

Since slight abnormalities in the pulmonary physical signs have such an important bearing on the early diagnosis of pulmonary tuberculosis, therefore, it is highly important to be acquainted with all the conditions under which such slight abnormalities may occur. Otherwise, we are not in a position to give them their proper diagnostic value in specific instances.

For many years we have been impressed, in making routine physical examinations of a large number of students and nurses, by the frequent

* Submitted for publication June 28, 1914.

occurrence of more or less marked apical abnormalities. On a number of occasions we have emphasized this experience in discussing the value of pulmonary examinations in the diagnosis of pulmonary tuberculosis. The matter has seemed to us of enough importance to make it desirable to have this experience in precise form, and with this object in view we undertook to study, with unusual care, the pulmonary physical signs in fifty healthy young adults.

For the purpose of this study we selected fifty students and physicians who, as far as symptoms go, were in perfect health. A minute examination was made of the chest by Dr. Hamman, and the results of inspection, palpation, percussion and auscultation were recorded and the deduction made therefrom as to the condition of the lungs committed to writing. A roentgenogram was then finished and reported on in writing by Dr. Baetjer, who had no knowledge of the outcome of the physical examination. Finally, the results of the physical examination and of the Roentgen-ray examination were compared.

The great difficulty in the way of satisfactorily appreciating slight deviations from the normal in pulmonary examinations is the absence of any concrete standard for comparison. The following brief statement of every-day observations illustrates what is meant.

When one looks at a well-developed man the chest is seldom seen to be quite symmetrical. Nearly always one clavicle is a little more prominent than the other, the corresponding shoulder a little lower, the upper dorsal vertebrae a little curved toward the opposite side, and on quite breathing expansion of the upper portion of the chest on that side is diminished. In right-handed individuals the changes are present on the right side, in left-handed individuals on the left side. The degree of difference varies greatly under what must be considered normal conditions, and there is no set limit to divide accurately the normal from the abnormal. If such changes are well marked on the side more used, they suggest the presence of pulmonary involvement on that side; if they are present on the opposite side, they point much more strongly to the presence of a lesion there.

Fremitus is a coarse sign of inestimable value in studying gross pulmonary lesions but lends little assistance in the discovery of slight changes. The quality of the voice and the character of the chest wall modify fremitus to such an extent that there is the widest individual variation. The sign is of value only comparatively between the two sides of the chest in the same individual. Such comparisons must take into account the normal accentuation of fremitus throughout the right side, particularly over the right upper. The difference is not a constant ratio, but varies greatly among individuals, and the difference must be very marked before we are justified in attaching to it diag-

nostic significance. When fremitus is felt better over the left upper than over the right upper, there is usually a lesion in the left upper lobe to explain the anomaly, although occasionally this difference exists in the absence of any other evidence of pulmonary abnormality.

In percussion we are forced again to a comparison of the note and sense of resistance in corresponding areas of the two sides of the chest. There is no absolute normal note with which to compare, the character of the note depending so largely on the thickness of the chest wall and the configuration of the chest. Our notion of the normal percussion note is a composite impression derived from a long and arduous experience in percussing innumerable chests. It is not an objective standard that we can demonstrate and measure by, outside of such experience. It is usually stated that the percussion note is a little less resonant above the right clavicle and in the right supraspinous fossa than at corresponding points on the left. We are not convinced that this difference exists normally to very light percussion. If a heavier percussion stroke is used the air in the trachea, which lies much nearer to the right than to the left apex, is thrown into vibration and the note is thereby modified.

The barriers in the way of standardizing physical signs are most obvious in considering auscultation. There is the greatest variation in the breath and voice sounds of normal individuals. In some, the breath-sounds are unusually loud, in others, unusually distant and faint; in some, expiration is more prominent at the apices than in others, and so on. Forced to use comparison of the intensity and quality of the breath and voice sounds at corresponding areas on the two sides, we face the difficulty raised by the normal difference between the two sides, particularly the well-marked difference at the apices. As in percussion, so in auscultation, there is no absolute normal and we must adopt an arbitrary standard that is born of individual experience.

These obvious difficulties are mentioned to emphasize the statement that this study is offered merely as a personal contribution to an interesting subject. What is found in the lungs on physical examination will vary with skill and experience, purely personal qualities, which give a strong individual flavor to the result. Still, we are convinced of the accuracy of our general conclusions and believe they are verifiable, although no two men might concur in detail about the interpretation of the physical examinations. We wish to emphasize further that we have sought for the very slightest deviation from normal in the physical signs, deviations that in routine pulmonary examinations we would disregard. But this will appear plainly enough in the illustrations we will furnish.

Having in mind the exception that might be taken to deductions based on examinations which we freely admit lack a desirable standardization, we sought to give them strength through the support of Roentgen-ray examinations. Roentgenograms are certainly objective, and though their skilled interpretation requires experience, still the data on which the conclusions rest can be satisfactorily demonstrated and are incontrovertible. A comparison of the results of the physical examination and the Roentgen-ray control must therefore reveal points of great interest and importance.

In reading the Roentgen-ray plates the following points were noted:

1. The density of the hilus shadow, which is recorded as normal, slightly increased, moderately increased or markedly increased.

2. The number and position of gland shadows.

3. The bronchial tree shadow (which includes bronchi, vessels and connective tissue), which is recorded as normal, slightly increased, moderately increased or markedly increased, and differences in various locations described.

4. The density of the lung substance itself. Special attention was given the condition of the apices and indeed abnormalities were limited mostly to the apices. On Roentgen-ray examination, the normal lung apices are clear and the apical spaces are equal on the two sides. The earliest change occurring at the apices is invasion by fine radiating lines extending from the bronchial tree, subdivisions which become visible only as the result of inflammatory changes. Following this the whole apical region becomes a little cloudy and opaque, probably due in large part to pleural changes. Finally, contraction may occur, which is early evidenced by narrowing of the space between the first and second ribs.

Regarding the Roentgen-ray findings as a control of the physical examination, the fourth point alone comes into consideration. Mediastinal shadows, though dense, give no physical sign. Glands enlarged sufficiently to cast definite shadows to Roentgen-ray illumination are still quite beyond the reach of percussion and auscultation. Increased density of the bronchial tree is discoverable only when the increase is unusually marked and extends close to the surface of the lungs in the region of the apices.

Reviewing the results of the physical examination we find that of the fifty healthy adults examined, in eleven the lungs are recorded as practically normal. We say practically normal, because in only five the note was made that absolutely no abnormality was found; in the remaining six very slight changes in the physical signs were discovered, but the changes were so slight that they were considered questionable. The five perfectly normal cases all had Roentgen-ray examinations. In

three the note is made, "Perhaps slight clouding at the right apex, but practically normal;" one showed very slight clouding at both apices; one, slight clouding and retraction at the left apex. An illustrative case is the following:

CASE 1.—Medical student, aged 23; weight 168 pounds; height 5 feet 11½ inches. No tuberculosis in the family. Always strong and healthy. Measles and mumps in childhood. Typhoid fever at 15 years of age. No pulmonary infections. Not subject to colds.

Well-nourished man of healthy appearance. Chest is well formed. Right clavicle a little more prominent than the left. Right shoulder sags a little. Viewed from in front, the right seems to move more than the left, but viewed from behind the left shoulder moves distinctly a little more than the right. (Right-handed.) Fremitus is better felt on the right in about the normal proportion. The percussion note is everywhere resonant, no definite difference between the two sides. Breath sounds are everywhere vesicular and clear-cut. Whispered voice sounds show the normal relation.

Apical resonance zone: right, 7 cm.; left, 7 cm. Diaphragm movements (difference of lung border on full inspiration and forced expiration), right, 9.5 cm.; left, 9.5 cm. Circumference of chest: at rest, 93 cm.; full inspiration, 99 cm.; full expiration 90 cm.

Impression: A normal chest.

Roentgen-ray plate 16.538. Moderately increased density of the hilus shadow on both sides. Numerous small fibrous glands on both sides. Slight thickening of the bronchial tree throughout on both sides. Perhaps very slight clouding at the right apex.

Of the six practically normal chests with slight, questionable physical signs, five had Roentgen-ray examinations. In all five physical examination indicated slight changes at the right apex, whereas Roentgen-ray examination showed normal lungs in one instance, slight changes at the left apex in three and slight changes at the right in one. The following is an illustration from this group.

CASE 2.—Medical student, aged 22; weight 158½ pounds; height 5 feet 10¼ inches.

No tuberculosis in the family. Has always been strong and healthy. Measles, chicken-pox, mumps and pertussis in childhood. No pulmonary disease. Not subject to colds or bronchitis.

Well-nourished man of healthy appearance. The chest is well formed. Right clavicle is a little more prominent than the left. Some restriction of movement over the right upper, front and back. The lower right moves a little more than the left. (Right-handed.) Fremitus is more marked on the right than on the left and the difference is perhaps a little greater than normal. The percussion note is a little different at the two apices, but it is difficult to say which side is abnormal. The impression is that there is a little impairment above the right clavicle and in the right supraspinous fossa. The breath sounds are clear cut and vesicular; they are unusually well heard on both sides; a little harsh and a little blowing above the right clavicle and in the right supraspinous fossa. The whispered voice sounds are well transmitted on both sides, the intensity on the right being a little greater than is usually heard. After coughing, a few transient crackles are heard above the right clavicle. A. r. z.: right, 6.5 cm.; left, 6.5 cm.; diaphragm movements in midaxillary line; right, 8.5 cm.; left, 8.5 cm. Circumference of the chest at the nipples: at rest, 83 cm.; full inspiration, 89 cm.; full expiration, 82 cm.

Impression: The chest is normal except for the slight changes at the right apex and these are questionable. The percussion note is perhaps a little impaired and the breath sounds a little more intense than they are usually heard.

Roentgen-ray plate 16,604. There is very slight increased density of the hilus shadow on the right side. No glands visible. The bronchial tree shows a normal degree of density. There is very slight clouding and definite, though slight retraction of the left apex. Diaphragm is normal. Heart pendulous.

Of the fifty examinations, in eighteen, definite abnormalities were found at the right apex; in fifteen, though definite, the abnormalities were slight, in three they were well marked. Of the fifteen with slight though definite changes in the physical signs the Roentgen-ray results accorded in detail with the physical examination in seven instances; in two instances slight changes were found at the left apex as well as at the right; in three the right apex was found clear, though there was a little clouding at the left apex; in two no definite changes were discovered in the lung; one had no Roentgen-ray examination.

The following is an illustration of agreement in the results of the physical and Roentgen-ray examinations.

Case 3.—Medical student, aged 23; weight 133 pounds; height 5 feet 8½ inches. No tuberculosis in family. Always strong and healthy. Mumps, measles, pertussis and croup in childhood. No pulmonary infections.

Well nourished, good color, healthy appearance. Chest is well formed; right clavicle a little more prominent than the left. The right shoulder sags somewhat. Movement a little restricted over the right upper. (Right-handed.) Fremitus better felt on the right than on the left in about the normal proportion. Percussion note definitely a little impaired above and below the clavicle and in the supraspinous fossa on the right. Above the right clavicle inspiration is obscured and distant, whereas expiration stands out prominently and is prolonged and blowing. The same relation exists but to a less marked degree below the clavicle and in the supraspinous fossa. On the left breath sounds are normally vesicular and clear cut. The voice sounds are better transmitted above and below the clavicle and in the supraspinous fossa on the right than is usual.

Apical resonance zone: right, 6 cm.; left, 7 cm. Diaphragm movements: right, 8 cm.; left, 8 cm.

Roentgen-ray plate 16,609. Moderate increased density of the hilus shadow on both sides, more marked on the right. No enlarged glands. Moderate increased density of the bronchial tree generally, more marked on the right than on the left. Slight clouding of the right apex above the clavicle. The right side of the diaphragm is a little irregular in outline.

The following instance illustrates disagreement between the physical and Roentgen-ray examination. It is possible that the spinal curvature may have caused the change in the physical signs.

Case 4.—Medical student, aged 27; weight 147 pounds; height 5 feet 8¼ inches. No tuberculosis in the family. Always strong and healthy. Measles and pertussis in childhood. No serious illness since childhood. Rather subject to colds. Has chronic catarrh of nose and throat.

Fairly well nourished. Healthy appearance. Marked retraction of the right side; the whole side apparently smaller than the left. Right clavicle

more prominent than the left, with marked retraction below the clavicle. Right shoulder droops and there is slight curvature of the spine to the left in the upper dorsal region. The scapula is low. Marked deficiency of movement on the right side. (Right-handed.) Fremitus is better felt on the right than on the left in about the normal relation. The percussion note is a little impaired above and below the clavicle on the right and perhaps a little impaired above the clavicle and in the supraspinous fossa on the left. Inspiration above the clavicle and in the supraspinous fossa on the right is a little obscure and expiration a little blowing. Above the clavicle and in the supraspinous fossa on the left breath sounds are a little harsh. The whispered voice sounds are more accentuated than normally above the clavicle and in the supraspinous fossa on the right.

Apical resonance zone: right, 6 cm.; left, 6 cm. Diaphragm movements: right, 8 cm.; left, 8.5 cm. Circumference of the chest at rest, 84 cm.; full inspiration, 91 cm.; full expiration, 82 cm.

Impression: Definite changes at the right apex and perhaps slight changes at the left.

Roentgen-ray plate 20641. Slight increased density of the hilus shadow on both sides. No glands. Very slight thickening of the bronchial tree on both sides. The lungs are normal. The diaphragm is a trifle higher on the left than on the right.

Of the three instances with more marked abnormalities at the right apex, two had Roentgen-ray examinations. In one the Roentgen ray and physical examinations agree in detail, in the other the Roentgen ray reveals a slight lesion on the left, besides the more marked change at the right. The latter instance is briefly as follows:

Case 5.—Medical student aged 22 years; weight, 160½ pounds; height, 6 feet ½ inch. No tuberculosis in the family. Has always been strong and healthy. Chicken-pox, measles and pertussis in childhood. Several attacks of bronchitis, one during the last few weeks.

Well nourished, good color, healthy appearance. Chest well formed, the right clavicle a little more prominent than the left. Slightly less movement over the right upper. (Right-handed.) Fremitus better felt over the right in about the normal proportion. Percussion note definitely impaired above the clavicle and less markedly so below the clavicle and in the supraspinous fossa on the right. Above the right clavicle inspiration is harsh and expiration prolonged and blowing; below the clavicle and in the supraspinous fossa expiration is a little more blowing than normally. Whispered voice sounds are better transmitted on the right than is usual. A few fine transient râles above the right clavicle.

Apical resonance zone: right, 6.5 cm.; left, 7 cm. Circumference of chest: at rest, 85 cm.; full inspiration 91 cm.; full expiration, 82 cm.

Impression: Definite changes at the right apex.

Roentgen-ray plate 16660. Moderate increased density of the hilus shadow on both sides. Few small fibrous glands on the right. Moderate increased density of the bronchial tree more marked on the right than on the left and particularly well marked in both upper lobes. Slight clouding of both apices, more evident on the right than on the left.

Twelve of the fifty examinations showed definite changes in the physical signs at the left apex; in eight instances the changes, though definite, were not marked; in four they were well marked. In the eight instances with definite though slight changes, the Roentgen-ray examination agrees accurately with the physical examination in three;

in four it reveals a slight lesion at the right as well as left; in one the left apex is found clear, the right a little cloudy.

In the four instances of more marked changes at the left apex the Roentgen-ray examination agrees accurately, except that in one slight clouding is present as well at the right. As an illustration of this group, the following instance is cited:

Case 6.—Physician, aged 27; weight, 162 pounds; height, 5 feet 11 inches. No tuberculosis in the family. Lobar pneumonia involving the right lower lobe five years ago. Typhoid fever eight years ago. Has mild attacks of bronchitis about once a year, the last attack about four months ago.

Well-nourished man of healthy appearance. Chest well formed, symmetrical. Clavicles a little prominent, the left a little more prominent than the right. The left shoulder sags a little. There is very slight curvature of the spine to the right in the upper dorsal region. Movement is diminished over the left upper. (Right-handed.) The fremitus is better felt on the right than on the left but the difference between the two sides is not as marked as is usual. The percussion note is a little higher pitched above the left clavicle elsewhere it is perfectly resonant and equal on the two sides. Inspiration above and below both clavicles is rather obscure and not as clearly vesicular as normally. Above the left clavicle inspiration has lost its sharp, crisp quality; in the left supraspinous fossa breath sounds are diminished in intensity. Above and below the clavicles and in the supraspinous fossa the whispered voice sounds, though better transmitted on the right than on the left, do not show in this respect as great a difference as is usual. After coughing there are a few transient crackles above and below the left clavicle.

Apical resonance zone: right, 6 cm.; left, 6 cm. Diaphragm movements: right, 8.5 cm.; left, 8.5 cm. Circumference of chest: at rest, 86.5 cm.; full inspiration, 92.5 cm.; full expiration, 84.5 cm.

Impression: The physical signs point toward a definite though slight lesion at the left apex.

Roentgen-ray plate 18163. Markedly increased density of the hilus shadow on both sides, especially on the right. A few small fibrous glands on the right side in the second and third interspaces. Bronchial tree shows slight thickening throughout both sides a little more marked on the left than on the right in upper lobe. There are definite small patches of fibroid tissue scattered throughout the right lower lobe. The right apex, however, is clear. There is definite clouding of the left apex. Diaphragm normal.

Comment: In this case the results of the physical examination and the Roentgen-ray examination almost completely coincide; perhaps the marked increased density of the bronchial tree in the right lower lobe may be the remains of the pneumonia five years ago.

In nine of the fifty cases there were abnormalities in the physical signs at both apices; in six the changes were definite though slight, in three very well marked. In the six with slight changes the Roentgen ray and physical examinations coincide in three; whereas, in the remaining three the Roentgen ray showed changes at the left apex alone. Of the three with well-marked changes two had Roentgen-ray examinations and in both they correspond accurately with the results of physical examination. These two individuals showed more marked

changes on both physical and Roentgen-ray examination than any others in this series and a brief account of the findings follows:

CASE 7.—Physician, aged 42; weight, 117 pounds; height, 5 feet 3½ inches. Mother died of tuberculosis when patient was 6 years old; one brother has had tuberculous pleurisy. Has always been healthy, but never robust. Had diphtheria at 6 years, no serious illness since. Several attacks of tonsillitis. Bronchitis at age of 19. No serious illness. Not subject to colds.

A small man of delicate appearance. Left clavicle a little lower than the right; viewed from in front the left side moves a little more than the right; viewed from behind the right shoulder moves definitely more than the left. (Right-handed.) Fremitus is better felt on the right than on the left side and the difference is greater than is usually present. The percussion note is definitely impaired above and below the right clavicle and in the right supraspinous fossa; there is definite but not marked impairment above the left clavicle. Above the right clavicle inspiration is distant and obscure; expiration is distant but definitely blowing in quality. On deep breathing inspiration is harsh and roughened above and below the clavicle and expiration quite blowing. In the right supraspinous fossa inspiration is harsh and halting; expiration blowing in quality but not accentuated. Above the left clavicle and in the left supraspinous fossa breath sounds are diminished in intensity, on deep breathing inspiration is very harsh. The whispered voice sounds are very definitely increased in intensity above and below the clavicle and in the supraspinous fossa on the right; they are also unusually well heard in the left supraspinous fossa. After coughing a few fine râles are heard above and below the right clavicle which subsequently disappear; and a few transient râles in the left supraspinous fossa.

Apical resonance zone: right, 5.5 cm.; left, 6.5 cm. Diaphragm movements: right, 6.5 cm.; left 7.5 cm. Circumference of chest: at rest, 75 cm.; full inspiration, 84 cm.; full expiration, 74 cm.

Impression: There is a very definite lesion in the right upper lobe and a definite though less extensive lesion in the left upper lobe.

Roentgen-ray plate 21079. Markedly increased density of the hilus shadow on both sides. Small number of fibrous glands on both sides. There is marked thickening of the bronchial tree throughout both lungs and especially marked in both upper lobes. Marked clouding of both upper lobes with retraction, especially well marked over the right upper lobe. Three small cavities in the right upper lobe and one in the left. Diaphragmatic adhesions on both sides.

CASE 8.—Student, aged 22. Weight, 127¾ pounds; height, 5 feet 6 inches. Father, mother, five brothers, all living and well. Two paternal aunts died of tuberculosis. Has always been well except for throat trouble; voice is often hoarse; clears throat frequently. Severe diphtheria in childhood. At the age of 10 and again at the age of 14 had severe grip which affected the throat. Measles mumps and pertussis in childhood. General health has always been good.

Fairly well-nourished man; skin a little sallow. Mucous membranes good color. The chest in general is well formed. The right clavicle is a little more prominent than the left. The right shoulder sags a little; slight limitation of movement over the right upper. (Right-handed.) Fremitus is better felt on the right than on the left side; indeed it is exaggerated on the right side. There is definite impairment of the percussion note above and below the right clavicle and in the right supraspinous fossa. Above and below the right clavicle inspiration has almost completely lost its vesicular quality and is very blowing, and expiration is intensely tubular in quality. The same changes are present in the supraspinous fossa, but not so marked as in front. Above and below the left clavicle expiration is better heard than normally, but inspiration is clear-cut and vesicular. Whispered voice sounds are

markedly increased in intensity above the right clavicle and to a less extent below the clavicle and in the supraspinous fossa. No râles.

Apical resonance zone: right, 6.5 cm.; left, 6.5 cm. Diaphragm: right, 7.5 cm.; left, 7.5 cm. Circumference of chest: at rest, 77 cm.; full inspiration, 84 cm.; full expiration, 75.5 cm.

Impression: A very definite and well-marked lesion in the right upper lobe; also a slight lesion in the left apex.

Roentgen-ray plate 20091. Markedly increased density of the hilus shadows, especially marked on the right. A few small fibrous glands on the right. Marked thickening of the bronchial tree especially below both clavicles; more marked on the right than on the left. There is marked clouding above and below the right clavicle but no retraction; slight clouding at the left apex without retraction. Diaphragm normal.

To state the results briefly: A careful physical examination of the chest of fifty healthy adults shows the pulmonary physical signs to be practically normal in eleven, definitely though not markedly altered in thirty, more obviously altered in seven and greatly changed in two. That the changes in the physical signs are the expression of corresponding changes in the lungs is borne out by the results of the Roentgen-ray examinations. Forty-six of the series had roentgenograms taken and in only six were the apices found to be normal.

The results of the physical and Roentgen-ray examinations, although agreeing in a large proportion of the cases, still not infrequently are at variance. In twenty-two of the forty-six cases the agreement is complete. The disagreement in the remaining twenty-four cases is as follows:

In six instances the physical examination shows practically normal conditions, whereas the Roentgen-ray examination shows slight apical clouding. This source of disagreement scarcely needs comment. To give such slight changes in the physical signs as are under consideration, a small lesion must be favorably located. A small lesion near the surface will give more obvious physical signs than a larger lesion more deeply situated, whereas the Roentgen-ray examination will reveal the condition of the apex as a whole.

In eighteen instances the physical examination indicates a definite lesion at one apex, whereas the Roentgen-ray examination shows clouding at both apices. This is hardly a serious disagreement.

In four instances the physical examination indicates a slight lesion at one apex whereas the Roentgen-ray examination shows clouding at the opposite apex. In all four instances the changes in the physical signs were very slight. One is at times sure that there is a little difference in the percussion note at the two apices, though it may be impossible to say which apex is normal and which changed. Also it is difficult at times when the breath-sounds vary to be sure that they are exaggerated at one apex rather than diminished at the other.

In two instances the physical examination indicates slight abnormalities at one apex whereas the Roentgen-ray examination shows both apices to be clear. One such instance is cited in the text, the difference in the physical signs being due, we believe, to the lateral scoliosis.

In four instances the physical signs indicate a lesion at both apices, whereas Roentgen-ray examination reveals clouding at only one apex. It is interesting that in all four instances the Roentgen-ray examination shows the lesion to be at the left apex.

Regarding the nature of the alterations in the lungs that occasion the changes in the physical signs and in the Roentgen-ray shadows, we are not prepared to make a definite statement. The increase in the hilus shadows, the glands and the deepening of the bronchial tree shadows, are regarded as evidence of old infections. No doubt tuberculous infections are frequently the cause, but tuberculosis leaves no specific marks and experience has convinced us that other infections, bronchitis, pneumonia, influenza, etc., may cause such changes and are indistinguishable from those produced by tuberculosis. The changes at the apices, infiltration, fibrosis and pleural thickening, we consider from general evidence to be nearly always the results of old quiescent tuberculous infections. The frequency with which such apical abnormalities are discovered in children under 12 years of age lends support to the view.

The important bearing that the facts here brought out have on the early diagnosis of pulmonary tuberculosis needs but hasty comment. That a large proportion of healthy young adults display evident apical abnormalities must have its weight in considering the value of such signs in diagnosis. Osler aptly compares slight abnormalities in the pulmonary physical signs with the systolic murmur commonly heard at the apex of the heart. When interest in the physical signs of cardiac disease was in the period of early enthusiasm an apical systolic murmur meant mitral insufficiency. Now that long experience has given these observations great precision, mitral insufficiency (meaning an organic mitral lesion) is not so lightly diagnosed on the isolated evidence of an apical systolic murmur. This does not imply that the presence of a systolic murmur is to be ignored, for it still has an important place in diagnosis, but it means that we have learned gradually to give it its just value as a part of the whole clinical picture. For instance, in acute rheumatic fever endocarditis occurs frequently and as it attacks the mitral valves more commonly than the others we often hear a systolic apical murmur as an early indication of mitral damage. However, experience has taught us that systolic apical murmurs are commonly heard in febrile conditions when there is not the

slightest reason for suspecting the presence of mitral endocarditis. Therefore, when in the course of rheumatic fever we observe an apical systolic murmur we immediately think of the possibility of mitral endocarditis, but we do not affirm its presence. The diagnosis between a so-called "functional" and a so-called "organic" mitral regurgitation depends on the evidence offered by the complete examination and the clinical symptoms. Indeed, with all available data at hand the differentiation is not always possible. Exactly analogous conditions exist in the relation of slight pulmonary apical abnormalities to the diagnosis of pulmonary tuberculosis. As isolated facts they by no means justify a diagnosis of pulmonary tuberculosis, although they acquire important value when supported by other evidence.